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THE UNIVERSITY OF ALBERTA

THE INFLUENCE OF SUB-TOTAL NEPHRECTOMY ON GLUCOSE METABOLISM IN RATS

by

© ABDUL MANNAN

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY
IN
BIONUCLEONICS

FACULTY OF PHARMACY AND PHARMACEUTICAL SCIENCES

EDMONTON, ALBERTA
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THE UNIVERSITY OF ALBERTA

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled THE INFLUENCE OF SUB-TOTAL NEPHRECTOMY ON GLUCOSE METABOLISM IN RATS submitted by Abdul Mannan in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Bionucleonics.

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External Examiner

Date 18 December 1973

DEDICATION

The loving memory of my father (whom I lost in my sojourn), the late PANDIT Moslehuddin Ahmad for the idealism he instilled in me and his uncompromising principles that have guided his entire life, and the devotion he exemplified as a dedicated teacher in the struggling life of a primary teacher.

My mother, SALEHA, for leading her children into intellectual pursuits through many sacrifices and hardships and patience by her strong determination and strict discipline and affection.

MONOWARA, my beloved, inspiring, and wonderful wife, for her unparalleled courage and conviction, patience and sacrifice, dutifulness and devotion, love and longing to her family through thick and thin.

Our very loving children: Ratan-Bulu,
Shaheen-Rosy
Akash-Faruk

for their amazing understanding, terrific faith and deepest love.

ABSTRACT

The influence of sub-total nephrectomy in male Wistar rats has been investigated. Three broad areas of study were undertaken:

i. SGOT, BUN, Serum CPK, plasma alkaline phosphatase, hematocrit, plasma and urinary glucose and plasma amino acids

ii. Hepatic glycogen (deposition and structure), hepatic and muscle glycogen cycle enzymes (amylo-1,6-glucosidase; glycogen synthetase, UDP-glucose:α-1,4-glucan α-4-glucosyl-transferase, EC 2.4.1.11; and glycogen phosphorylase, α-1,4-glucan:orthophosphate glucosyl-transferase, EC 2.4.1.1) and hepatic G-6-PDH

iii. The *in vivo* oxidation of ^{14}C-glucose to $^{14}CO_2$ and *in vivo* conversion of ^{14}C-glucose into liver and plasma protein, and muscle, liver and adipose tissue lipid and plasma FFA.

Sham operated rats and rats with restricted diet intake (i.e. rats given equal amounts of diet daily as usually voluntarily eaten by uremic rat) were used as controls; the latter to delineate the effects of starvation and caloric deficiency from the effects of uremia. Sub-total nephrectomy was found to cause the following abnormalities or derangements:

1. a decrease of SGOT activity
2. the appearance of 3-methyl histidine in

plasma, and elevation of plasma phosphoserine and 1-methyl histidine

3. hypoglycemia
4. abnormal hepatic glycogen deposition
5. alteration of the intra-molecular structure of hepatic glycogen, notably with increased 1,6-bonds (by 70%), segments (by 68%) and non-reducing ends (by 67%), but decreased glucose residues per segment (by 44%) with a visible deposition of glycogen in liver EM
6. an inverse relationship between hepatic amylo-1,6-glucosidase activity and glycogen synthetase, decreased, or normal hepatic phosphorylase activity, increased hepatic G-6-PDH activities, and increased muscle phosphorylase activity
7. a shift in glucose utilization patterns by channeling preferentially a large proportion of glucose from peripheral reserves for enhanced production of tissue protein and lipid

8. increased glycolysis above the G-6-P level, with a more active pentose monophosphate pathway and decreased gluconeogenesis, associated with increased lipogenesis.

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The Sacrifices of the scores of rats for the welfare and sustenance of mankind and glory of Science will ever be remembered.

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CHAPTER I

INTRODUCTION

The measurement of blood urea nitrogen (BUN) to monitor the progress of the chronic uremic condition is an accepted clinical approach; however, elevation of the BUN above normal occurs only when at least 25% of normal kidney function is lost. Moreover, the BUN can also be raised by stress, dehydration, and other physiological conditions. Therefore, it is imperative that an alternate parameter should be searched for, a parameter which can predict the early onset of uremia. Impaired carbohydrate metabolism in uremia is also a clinical fact. The biochemical sites of this effect, and the mechanisms involved are not yet clear.

This investigation of the influence of sub-total nephrectomy in rats include a study of:

A. SGOT, serum CPK, PAP, hematocrit, and plasma glucose, in a search for an alternate parameter which might monitor the very onset of nephron failure.
B. The deposition of glycogen in the liver, as revealed by EM and biochemical isolation.
C. The Structure of the liver glycogen.
D. Glycogen cycle enzymes.
E. The hexose monophosphate shunt.
F. The plasma amino acid profile.
G. *In vivo* oxidation of ^{14}C-U-glucose to $^{14}CO_2$ and

in vivo conversion of ^{14}C-U-glucose to glycogen, lipid and protein.

In chronic renal fialure, loss of appetite is known to influence the development of the clinical syndrome. Therefore, to dilineate the influence of sub-total nephrectomy in rats, the effects of diet restriction in normal rats were also studied.

CHAPTER II

LITERATURE SURVEY

A. Structure and Function of the Kidney

1. Structural division relating to function:

Kidneys are bilateral and retroperitoneal structures. In humans, each weighs 115 to 150 g, whereas the rat kidney weighs from 2 to 3 g. On Sagital section it can be seen that the kidney contains an outer cortical area or cortex (70%) and an inner medullary portion or medulla (30%). The cortex consists of the glomerules, proximal tubules, and distal tubules. The medulla consists of the loop of Henle, the vasa recta (a bundle of straight vessels formed by efferent arterioles of capillary dimension to dip deeply into medulla) and collecting ducts (see figures 1, 2).

2. Major functions of the kidney

The two major functions of the kidney are excretory, by means of which it adjusts and regulates the composition and volume of the body fluids, and non-excretory, by means of which it regulates biological systems and functions (notably red blood cell formation and possibly blood pressure). The excretory function is largely interwoven with the structure of the kidney. Each kidney has approximately one million nephrons, each nephron

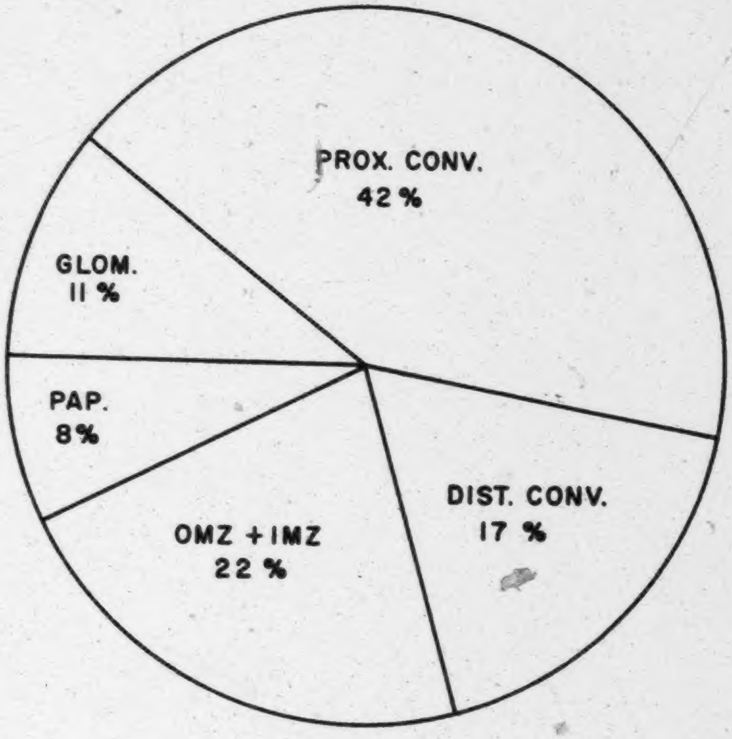


Fig. 1. Relative mass of the various structures and zones of the human kidney. (H. Mattenheimer, "Enzymology of Kidney Tissue," in Enzymes in Urine and Kidney, Ed. by U.C. Dubach, 1968, Hans Huber Verlag.
(Glom = glomeruli; Prox. conv. = proximal convoluted tubules; Dist. conv. = distal convoluted tubules; Pap = papilla; OMZ = outer medullary zone; IMZ = inner medullary zone).

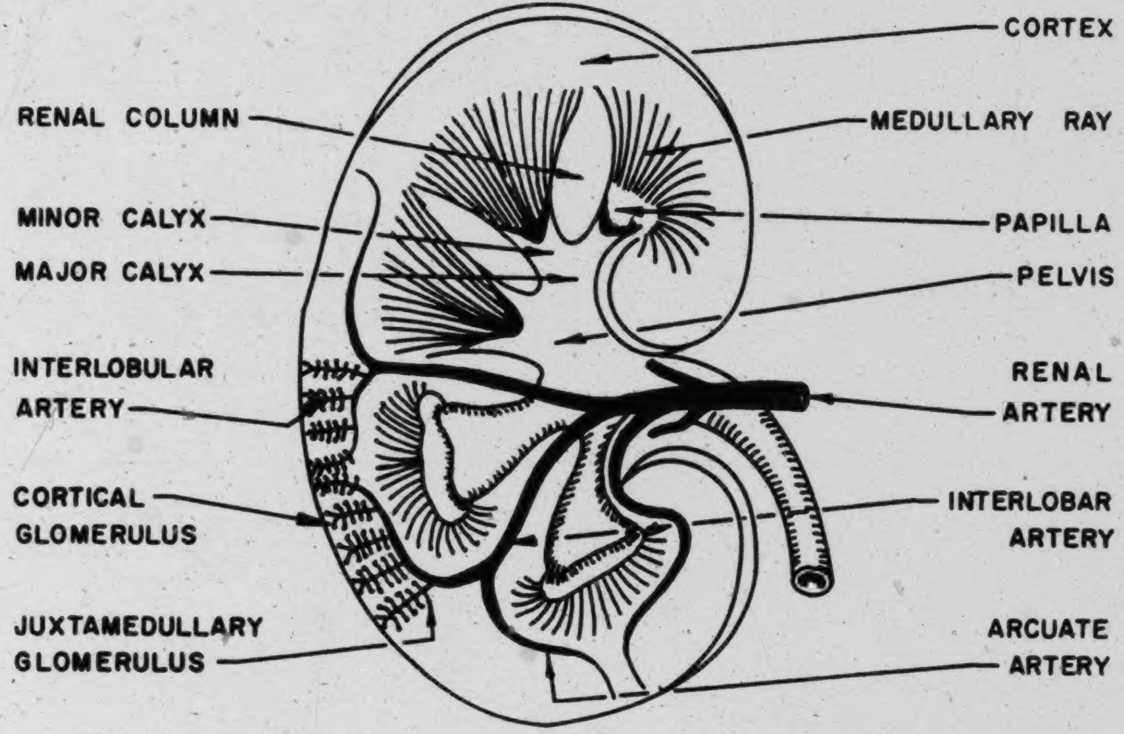

CORTEX
RENAL COLUMN
MEDULLARY RAY
MINOR CALYX
PAPILLA
MAJOR CALYX
PELVIS
INTERLOBULAR ARTERY
RENAL ARTERY
CORTICAL GLOMERULUS
INTERLOBAR ARTERY
JUXTAMEDULLARY GLOMERULUS
ARCUATE ARTERY

Fig. 2. Sagittal section of the kidney. The upper half depicts the overall gross anatomic arrangement. The lower half demonstrates the arterial supply.

consisting of the glomerulus, the proximal convoluted tubule (pct), the loop of Henle (descending and ascending) and the distal convoluted tubule (dct). The distal tubule enters a collecting duct which terminates in the calyceal system, draining other nephron units in its course through the cortex and the medullary pyramids (see figure 3).

The capillary surface of a glomerulus (which does the filtration) is about 1.5 cm^2. The blood flow through the human kidney amounts to 1,200 ml (∿700 ml plasma) per minute; about 1/5th of this, that is, 120 ml are filtered per minute. Therefore, the GFR is 120 ml/minute or 20% of the RPF. This is the primary step in the formation of urine. Only 1 to 2 ml of final urine are excreted by the kidney per minute, which means that of about 170 liters of fluid filtered per 24 hours, 99% is reabsorbed. Approximately 1,000 g of sodium chloride, 360 g of sodium bicarbonate and 170 g of glucose are reabsorbed together with water, while a total of 50 to 60 g of solids such as urea and other metabolites are excreted in 1 to 1.5 liters of urine.

Most of the blood supplied to the kidney flows through the glomeruli before it reaches the capillary network surrounding the tubular tissue. Any restriction of the blood flow through the glomeruli reduces the blood supply of the tubules and, if prolonged, causes degenerative changes of the tubular cells (as in the case of glomerulonephritis).

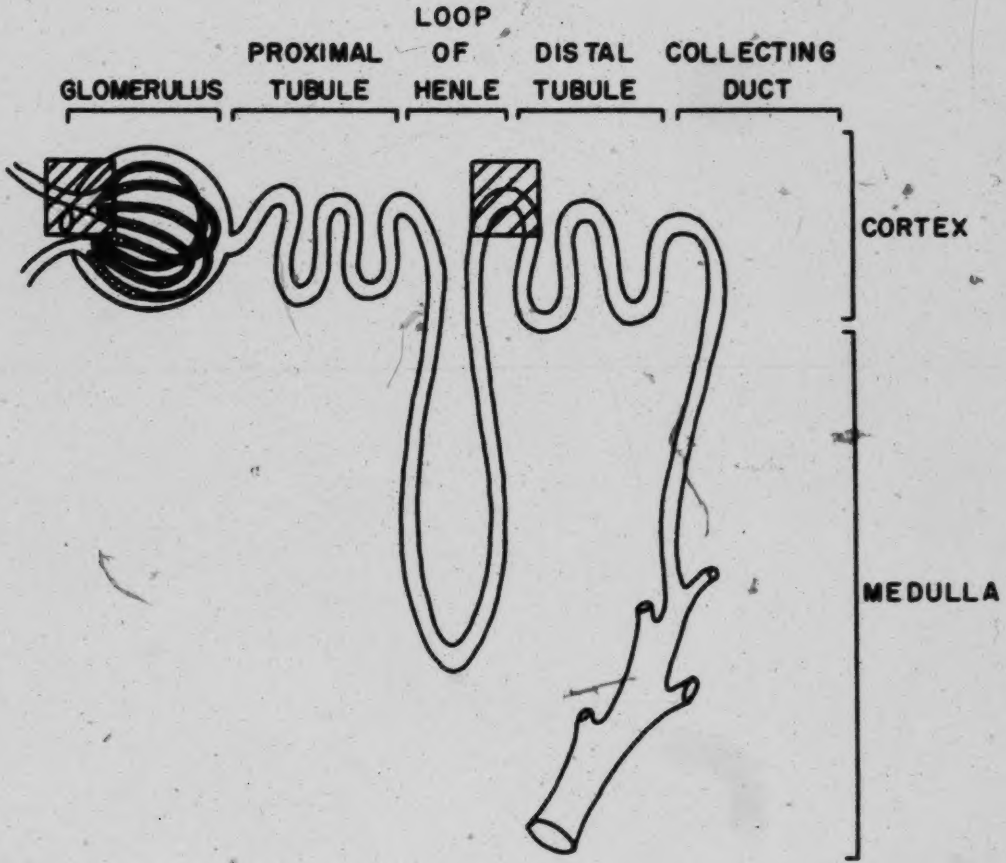

GLOMERULUS
PROXIMAL TUBULE
LOOP OF HENLE
DISTAL TUBULE
COLLECTING DUCT
CORTEX
MEDULLA

Fig. 3. A single, "elongated" nephron. The hatched squares indicate the areas that overlap in life to form the juxtaglomerular apparatus.

The colloid osmotic pressure of the blood reaching the tubules is increased because 18 to 20% of the plasma water was filtered in the glomeruli. The increased colloid osmotic pressure facilitates the reabsorption of water in the medulla. The force required for ultrafiltration is derived from the heart. The glomerular hydrostatic pressure of 60 mm Hg is opposed by colloid osmotic pressure of 30 mm Hg and a capsular hydrostatic pressure of 5 mm Hg, resulting in a net filtration pressure of 25 mm Hg.

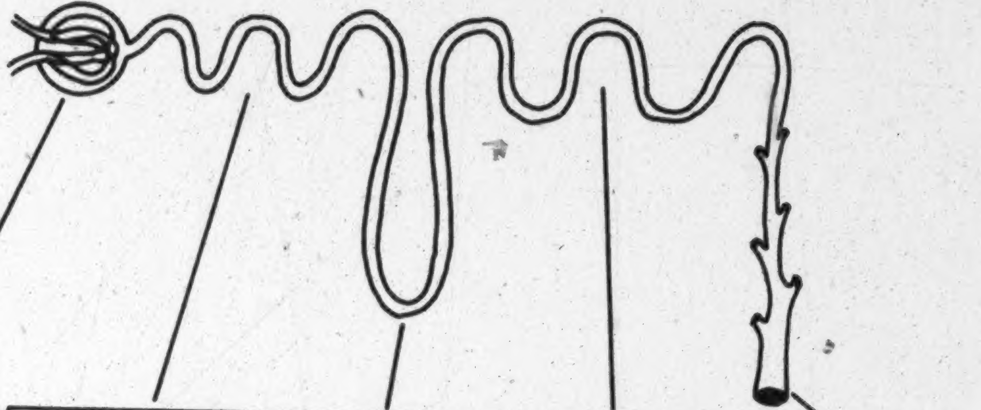
The production of urine is the result of three processes taking place in the nephron. The first is glomerular filtration, which depends on the effective filtration pressure which is the difference between the capillary blood pressure and the sum of oncotic pressure and capsular pressure (Bowman's capsule). The second process is the reabsorption of water and solutes in the various parts of nephron. Finally, there is secretion of solutes into the tubules.

Both reabsorption and secretion are controlled by active transport mechanisms (movement of substances against concentration or electrochemical gradient) which require energy as ATP which is generated in the tubular cells (mitochondria near capillary walls) by the enzymes of intermediary metabolism (lower activity in the glomeruli than in the other parts of the nephrons).

Sites of reabsorption and secretion in the nephron as identified by micropuncture technique are shown in figure 4. The proximal tubular cells have a brush border and an enlarged surface which enhance reabsorption; the fluid passing through remains isosmotic with plasma. In the loop of Henle, the isosmotic urine volume gets reduced before entering the loop (which in some nephrons is very short while in others, especially those originating from the juxtra medullary glomerulus, it is long and dips deeply into the medulla.

The medulla conserves water by a concentration mechanism through these loops and the vasa recta and maintains a concentrated medullary interstitium surrounding the collecting duct. This conservation is accompanied by the movement of Na^+ from inside the lumen of the thick ascending portion (cuboidal cells) of the loop to the interstitial area without accompanying water. Final concentration occurs by movement of water along a concentration gradient (interstitium). The vasa recta then removes this water, maintaining the high concentration of the medulla.

Distal tubules (with cuboidal cells which are smaller than proximal tubular cells and without a brush border) and collecting ducts (containing similar cells and sharing many functions of the distal tubules) are referred to as distal nephrons.



GLOMERLUS	PROXIMAL TUBULE	LOOP OF HENLE	DISTAL TUBULE	COLLECTING DUCT
Filtration	65% Na^+ & H_2O reabsorbed. ADH not required. All glucose, K^+, urate reabsorbed. HCO_3^- reabsorbed. H^+ secreted. Fluid leaves isotonic. Active reabsorbtion	Na^+ transport from ascending limb. Countercurrent establishes hypertonic interstitium. Fluid leaves hypotonic.	Na^+ reabsorbed. ADH required for H_2O reabsorption. K^+ & urate secreted. HCO_3^- reabsorbed. H^+ secreted. NH_3^+ secreted. Leaves hypotonic or isotonic. Passive reabsorbtion	Water reabsorbed in final concentrating operation. ADH required.

Fig. 4. Major functions of each portion of the nephron.

In the distal tubule uric acid, and potassium are secreted. Though acidification starts in the proximal tubule, the distal tubule also secretes H^+ and produces NH_3 to enhance the buffering capacity, allowing more H^+ to be secreted.

Final concentration of urine takes place in the collecting duct where membrane permeability to water is controlled by ADH (secreted by the hypophysis). Water moves from the collecting duct to the interstitium along the concentration gradient created by high medullary osmotic pressure, with some reabsorption of sodium in this region. Thus, the distal nephrons make the final adjustments in volume, concentration and pH of urine.

Carbohydrate, amino acids, fatty acids, and a number of intermediary metabolites of glycolysis, the hexose monophosphate shunt, and the TCA cycle are metabolized by the cortex tissue, while the medulla mainly utilizes glucose both in glycolysis and in the hexose monophosphate shunt. The TCA cycle is not functional in medullary metabolism.

The cortex carries out metabolism aerobically, while in the medulla, metabolism is anaerobic. Gluconeogenesis is potentially high in kidney (1,2,3,4,5,6,7,67).

3. Kidney Enzymes

Enzymes of all major and special metabolic pathways are found in the renal tissues (8). Activities of LDH, MDH, GlDH, GOT, SPT, and CA are lower in the glomeruli, while those of LDH, and GlDH are highest in the pct; those of GOT and CA are maximum in the dct, while MDH and GPT are highest in the medullary rays. G-6-PDH is highest in glomeruli, indicating a relatively high activity of the hexose monophosphate shunt.

(a) Ammonia Excretion

Plasma glutamine is a major precursor of urinary ammonia. Glutaminase I, activated by phosphate, splits glutamine to ammonia and glutamate; glutaminase II, activated by pyruvate or 2-oxo-acid hydrolyses the amide to ammonia and glutamate (figure 5) (9).

High glutaminase activity in the cortex and low activity in the medulla and papilla suggest that ammonia may be added to the urine in the cortical convolutated tubules. Glutaminase activity increases with increased excretion of ammonia in rats. Excretion of ammonia must always be accompanied by the excretion of H^+ ions to form NH_4^+, as ammonia diffuses freely through the cell membrane while NH_4^+ does not. To establish a high concentration gradient between urine and intestinal fluid, NH_4^+ must be formed in urine. Carbonic anhydrase (CA) catalyzes the formation of H^+ ions:

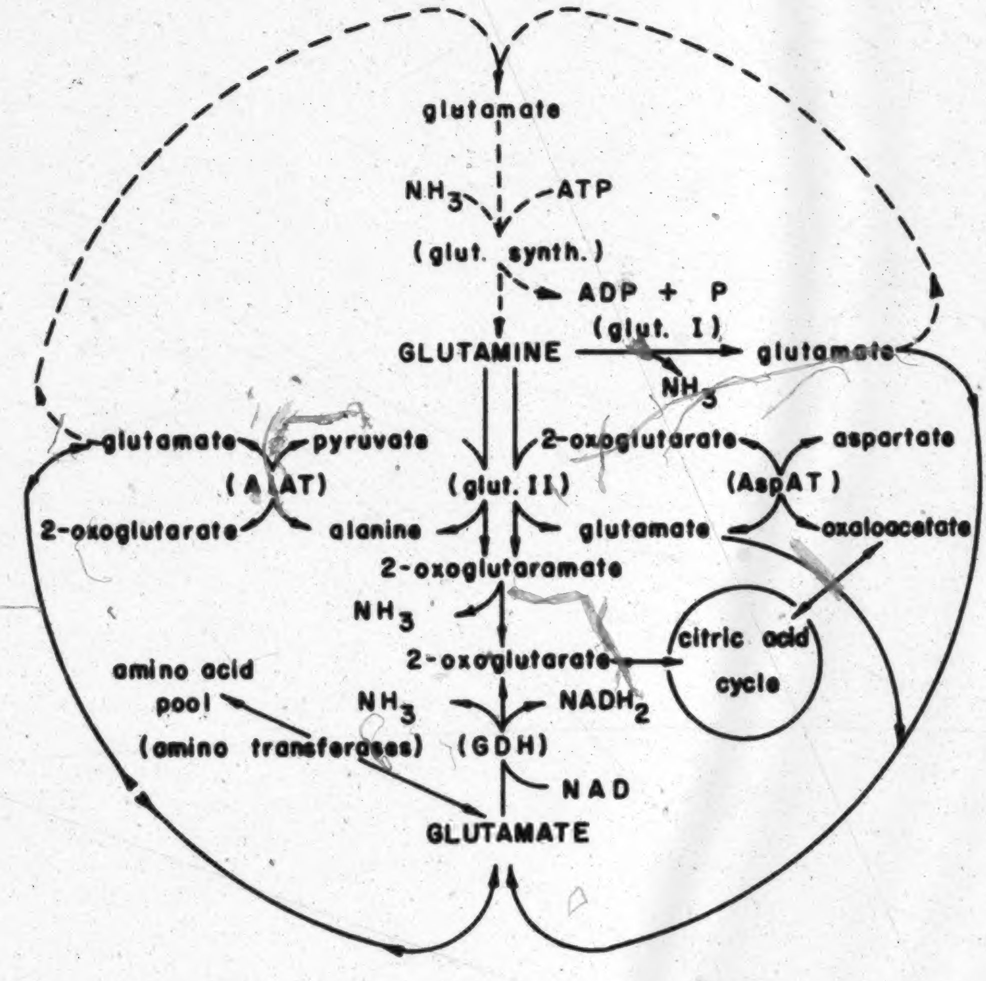

glutamate
NH3
ATP
(glut. synth.)
ADP + P
(glut. I)
GLUTAMINE
glutamate
NH3
glutamate
pyruvate
(AlAT)
2-oxoglutarate
alanine
(glut. II)
2-oxoglutarate
aspartate
(AspAT)
glutamate
oxaloacetate
2-oxoglutaramate
NH3
2-oxoglutarate
citric acid cycle
amino acid pool
NH3
NADH2
(amino transferases)
(GDH)
NAD
GLUTAMATE

Fig. 5. Interrelationship of the reactions involved in ammonia metabolism in the kidney. As there is doubt about the glutamine synthetase activity in the dog kidney, the reactions involved are represented by a dotted line. (V.E. Pollak *et al.*, J. Clin. Invest. 44, 169 (1965). American Society for Clinical Investigation, Inc. AspAT = GOT, AlAT = GPT, GDH = GlDH.

$$CO_2 + H_2O \xrightleftharpoons{CA} H_2CO_3 \quad H^+ + HCO_3^-$$

In the rat CA is inhibited by acetazolamide. This does not diminish renal ammonia production but decreases ammonia excretion by diverting ammonia to renal venous blood. CA mediates both H^+ excretion and reabsorption of filtered HCO_3^-. ~~CA~~ has been shown by micropuncture to be localized in the luminal membrane of proximal tubular cells and inside the cells of proximal and distal tubules. The decrease of pH occurs slightly more in distal segments of the proximal tubule and greatly in collecting tubes.

(b) Dehydrogenases and Amino Transferases

Aminotransferase contributes to ammonia production via glutamate dehydrogenase and also by amino acid reabsorption. LDH isoenzyme patterns differ significantly in the nephron from species to species. In the rat LDH_I (heart type) prevails in tissues with aerobic metabolism (highest in the cortex) and LDH_5 (muscle type) is most abundant in tissues with anaerobic metabolism (highest in the medulla).

(c) Renal Enzymes in Tubular Dysfunction

In tubular acidosis, the kidney loses its ability to excrete an acid urine. The excretion of ammonia is reduced and the urine contains bicarbonate, probably due to a deficiency of CA.

In hypokalemia, LDH activity in the pct and the dct increases due to impaired ATP synthesis, and glycolysis may become an alternate pathway for ATP synthesis. This is supported by the fact that in kidneys of hypokalemic rats the Pasteur effect (inhibition of glycolysis by oxygen) cannot be shown.

4. Urinary and Serum Enzymes

The kidney is the source of urinary enzymes. About 30 enzymes including oxydoreductases, transferases, hydrolases and lysases have been detected in urine, of which only a few enzymes (e.g. LDH, alkaline phosphatase, β-glucuronidase, catalase and LAP) have been investigated to monitor renal function. Other potential sources of urinary enzymes are erythrocytes, and leucocytes in pathological conditions (10). Lack of specificity, inappropriate negatives, increased values in the absence of disease and wide day to day fluctuations in excretion are major problems in monitoring these enzymes (10,11). In acute renal failure, elevated urinary enzyme concentrations are reported as a result of tissue damage. Kemp et al. (12) reported elevation of serum LDH, and aspartic and alanine transaminase in early phase of the anuric episode. In CRF, Ringoir et al. (13) reported elevation of the total LDH and LDH_5 activity in post dialysis blood. Eschar et al. (14) reported an increase in serum creatine phosphokinase in 43% of uremic patients and 50% of these patients after peritoneal dialysis.

B. Renal Function Tests

1. Qualitative Tests

(a) Blood Examination for BUN and Creatinine

Renal function in man and animals is commonly evaluated by the determination of blood urea. Fifty percent of serum NPN is contributed by urea, the remainder being creatinine, creatine, uric acid and amino acids.

As renal function diminishes, the NPN rises slowly until GFR is reduced to 50%, after which blood NPN levels become significantly greater than normal values. It has been known that serum urea levels inconsistently rise or remain near normal even after acute or long term damage to the kidney (15-18). Others have found that blood urea can be raised by conditions like stress, dehydration, circulatory failure, tissue necrosis, and gastrointestinal bleeding (19).

The most important fact is that when renal function is less than 33% of normal, serial estimations of elevated creatinine and BUN are relied upon increasingly as the urine approaches isosthenuria. At this stage creatinine clearance and PSP excretion (or the rate of disappearance of other secreted substances, such as PSP from blood) are most reliable. The ratio of BUN to serum creatinine (10:1) may be very useful in differential diagnosis (20).

The functional reserve of the kidney has been established by the relationship between plasma urea and GFR as follows:

$$Q = K \times GFR \times P$$

where GFR = glomerular filtration rate,
P = plasma urea level,
= concentration of urea in glomerular filtrate,
K = a proportionality constant.

In acute renal failure (ARF) the correlation between the severity of the illness and the BUN level applies more closely than it does in chronic renal failure (CRF). In CRF the plasma creatinine concentration (an index of muscle mass) seems to offer a better index of the degree of renal failure than BUN, particularly when the patient is on a low protein diet (21).

(b) Serum Enzymes

Estimation of serum enzymes is an insensitive method of assessing renal function. Karl et al. (22) found no change in G-6-Pase, G-6-PDH, GOT and glutamic pyruvic transaminase in rats with protein-urea, tubular dilution, and fragmentation of the brush border, but observed slight depression of alkaline phosphatase. Bealty et al. (23) found that serum enzyme patterns varied only slightly from control when kidneys were damaged either by-crushing, CCl_4 or anoxia; serum LDH slightly increased but esterase and MDH showed no change.

(c) Examination of the Urine for Protein

Increased output of urinary protein indicates an increased concentration in the glomorular filtrate, decreased reabsorption, or its diffusion from damaged tubular cells into the urine. Protein excretion is lower in tubular than glomerular damage. "Tubular proteinurea" is characterized by a predominance of β- and γ- globulins, and increased activity of urinary lysozyme and ribonuclease due to a decrease in tubular protein reabsorption.

(d) Urinary Enzymes

As indicated earlier, elevations of urinary enzymes are non-specific (24,25). Elevation of β-glucuronidase, for example, indicates pyelonephritis rather than infection of the lower urinary tract (26). Nonetheless, serial measurement of ULDH and UAP are considered to be useful in evaluating the course and type of glomerulonephritis (26). ULDH, UAP and lysozyme are considered to be aids in the diagnosis of kidney transplant rejection (27). Lysozyme excretion was found suitable for the clinical diagnosis of impaired renal tubular function (28). Catalase is used as a screening test for urine (29). Acid phosphatase has been suggested as a test for unilateral renal disease (30).

UGOT has no source other than tubular cells, and Mason et al. (31) proposed its use to study renal damage. Dunn et al. (32) found no elevated UGOT in animals with hepatic or myocardial damage or by injection of that enzyme, and Chinsky et al. (33) found only a small rise in UGOT in patients with high serum levels.

(e) Excretion Tests

i. The phenol red test (PSP; 0.1 mg in saline per Kg body wt) requires determining the per cent excretion in 30 minutes or 120 minutes. Seventy-five per cent of the dye is bound to plasma protein and 70% is extracted from the blood during a single passage through the kidney, partly by glomerular filtration but mostly by tubular excretion (34,35,15,36).

ii. At low blood levels, the excretion of PAH is a measure of renal plasma flow (RPF).

2. Quantitative Tests

(a) Clearance Tests

The term was first used by Möller for the excretion of urea (37), and then by Jolleffe et al. (38) for the excretion of creatinine to determine the rates at which kidneys excrete various substances in relation to their concentration in plasma. These methods are applied to estimate GFR, RPF and maximum excretory and absorptive capacities of the kidney tubules (Tm) (39).

GFR is measured by using an inert substance which will be completely filtered, and not be metabolized nor be reabsorbed or secreted by tubules. Inulin is an ideal substance, but complicated chemical analysis presents difficulties in its clinical use. Endogenous creatinine clearance determination is best.

The clearance of creatinine represents the volume of plasma cleared of it in 1 min by the kidney, and can be calculated from the concentration in the plasma (P), the concentration of urine (U) and the volume (V) of urine passed in 1 minute using the formula:

$$C_{Creat.} = \frac{U \times V}{P}$$

If the clearance of a substance is less than that of inulin then it is reabsorbed by the tubules; if the clearance is more than that of inulin then it is secreted by the tubule. Since inulin clearance represents GFR, clearance indicates measurement of RPF and GFR.

Qualitative aspects of renal function are related from IVP and the uptake of ^{203}Hg-chlormerodrin (40) by determining the amount passed in urine in 24 hours following an i.p. dose, and the amount retained in kidney tissue biopsy or autopsy. The results are expressed as the ratio of counts per g tissue to counts per ml of urine. Normally, the ratio runs from 2 to 6, and increases with the degree of renal damage; another radioisotopic test measures renal hippuran uptake, concentration and excretion after a single i.v. injection (Hippuran renogram). This gives a sensitive comparison of one kidney with the other and is a qualitative test of renal function (41).

Internal distribution of blood flow is estimated by the disappearance from the kidney of a dissolved radioactive gas (Krypton) (42). Renal artiography is more sensitive (43) but visualization of blood flow can be obtained after injection of $Na^{99}TcO_4$ (sodium pertechnitate), using a γ-Camera.

(b) The Test for Isosthenuria (Loss of Kidney Capacity to Concentrate or Dilute Urine)

This can be done by measuring the specific gravity and osmolality of urine (U_{sp} and U_{osm}). To test the diluting capacity, large quantities of water (20 ml/Kg body weight, every 30 minutes) are given and hourly urine volume and osmolality measured. At other times the patient goes without water for 12 to 24 hours and the maximum concentration of urine is measured.

Normally the volume of urine formed each day is 1 liter per 170 liters of water filtered. In acute renal failure (e.g. oliguria, anuria) this may fall to zero. The normal specific gravity of urine is between 1.002 to 1.045, depending on the type of substance excreted and the osmolality.

3. Sensitivity of the Tests

(a) Proteinurea is the indication of glomerular damage.

(b) The phenol red secretion test is less sensitive than other tests.

(c) The clearance tests are tedious to perform and results cannot be interpreted in terms of glomerular damage, tubular damage, or renal flow when the kidneys are damaged, and these tests are no more sensitive than the simpler concentration tests.

(d) The dilution test is less sensitive than the concentration test to indicate renal functional abnormality (but reduction in food intake especially protein intake may affect the concentration test) and papillary necrosis.

(e) The C_{PAH} is the quantitative test for proximal tubular function.

(f) Concentration and dilution tests measure medullary function (loop of Henle, and collecting ducts).

4. Diagnostic Tests and Normal Values

(a) U_{sp}: normal values: morning = 1.02. In ARF increases (concentration)
In CRF decreases (dilution).

(b) U_{mos}: normal: 800 to 1,350 mosM/Kg
In ARF increases
In CRF decreases

(c) BUN: normal: 7.2 to 20.2 mg/100 ml
In ARF increases
In CRF decreases
Rises when normal function decreases by more than 20%.

(d) Serum creatinine (more reliable than BUN). No appreciable rise will be recorded until over 60% of the glomeruli are destroyed.
normal: 1.19 mg/100 ml (men)
0.96 mg/100 ml (women)

In ARF increases
In CRF increases

(e) U creatine: normal: 140 ml/min (men)
112 ml/min (women)
In ARF decreases
In CRF decreases

(f) Electrolytes: serum electrolyte content varies depending on the cause of renal failure, the degree of hydration, and the diet. The majority show certain trends and these tests are very important.

normal: milliequivalents/liter (44)

Cations:	Na^+	142
	K^+	5
	Ca^{++}	5
	Mg^{++}	3
		155
Anions:	HCO_3^-	24 - 27
	Cl^-	100 - 103
	$HPO_4^=$	2
	$SO_4^=$	1
	Organic Ac^-	6
	Protein	16
		155

The predominant cation is sodium; protein regulates osmotic pressure; bicarbonate increases in alkalosis and decreases in acidosis.

In ARF generally there is an increase of NaCl (hypernatremia), Mg^{++}, K^+ (hyperkalemia) and Ca^{++} but in CRF NaCl, K^+, are normal or slightly elevated, Mg^{++} is elevated and Ca^{++} is decreased. Plasma and urinary pH measure distal tubular function. This is done by the ammonium chloride loading test.

5. Diuretics

Norepinephrine, caffeine, theophyllin and others lead to an increase of arteriol pressure of the kidney with an increase of GFR. Urea, mannitol, sucrose, etc., increase tubular osmotic pressure to prevent reabsorption. Other diuretics such as chlorothiazide decrease antidiuretic hormone.

C. Nephron Failure

It has been estimated that 20 to 40 people per million of population below the age of 60 will present each year with irreversible renal failure. In the U.S.A., 4,000 to 8,000 people present for dialysis each year. In Canada, 5,000 people suffer from uremia each year. No statistics are available for the developing and undeveloping countries.

1. Definition and Etiology

Renal failure or impaired kidney function due to failure of nephrons, the functional units are of two types:

(a) Acute Renal Failure (ARF)

Rapid and severe reduction of renal excretory function (e.g. decreased GFR - normal 125 ml/min; olguria - decreased urine; anuria - absence of urine secretion) may be due to:

i. prerenal causes (decrease of cardiac output due to trauma, shock hypoxia, necrosis).

ii. post renal causes (obstruction of the ureters (i.e. obstructive uropathy leading to irreversible parenchymal damage).

iii. renal causes due to distortion of renal architecture either in tubular or cortical necrosis or tubular blockage or disease of the glomuruli. Trauma, cardiac failure, bacterial endotoxins and extensive burns lead to a decrease in blood volume and then to a decrease in cardiac output giving rise to renal vasoconstriction. This causes renal ischemia, disrupting tubular integrity and necrosis. In the course of time, with care and treatment, it may be reversed.

(b) Chronic Renal Failure (CRF)

This is a clinical state of progressive destruction of nephrons (irreversibly) over many months or years until the kidney no longer functions. CRF has the same effects on body fluids as ARF, but these effects are less severe. In CRF the commonly occurring renal causes are:

i. Arteriosclerosis (i.e. disease of blood vessels thickening up).

ii. Pyelonephritis, a progressive destruction of the structure of renal tubules and glomeruli by invading bacteria, abscesses, polycysts (a hereditary disease) or trauma (45).

D. Uremia and the Urea Cycle

The term uremia, meaning urine in the blood, was introduced by Piorry in 1840 and l'He-ritier. Renal failure was regarded as a form of poisoning of the blood due to reabsorption of urine. Now, the term uremia is used clinically to describe the state principally associated with the retention of nitrogenous metabolic products; the condition is characterized by an elevated blood urea concentration, though the elevation does not necessarily correlate with other aspects of renal insufficiency (1).

J.P. Perers (46) in 1935 wrote "the kidneys appear to serve as the ultimate guardians of the constitution of the internal environment". The kidney carries out its homeostatic functions by the process of glomerular filtration, tubular reabsorption and secretion and thus regulates the concentration of metabolic end products, the osmotic pressure, the volume, and the ionic composition of the internal environment. In renal failure, the end results are the alterations in the constitution of the internal environment, the fundamental biochemical significance in the sustenence and maintenance of life.

1. The Role of Urea and Other Toxic Metabolites

Urea is formed only in the liver and may be regarded as the end product of protein catabolism. It is distributed throughout the total body water having equal concentration in the intracellular and extracellular fluid. Thus its concentration is the same in whole blood as in serum. In non-acute disease, and in health, the rate of excretion of urea is equal to its rate of production in the body. It is excreted by glomerular filtration, although half of the amount filtered diffuses back into the blood through the tubules. The relation between BUN and GFR is such that at any given rate of urea production their product remains constant (to keep rate of excretion equal to rate of production).

$$\text{BUN} \times \text{GFR} = K$$

It can be seen from figure 6 that the BUN is not a very precise measure of renal function, because before the BUN rises above normal a considerable deterioration of renal function must be present. At normal rates of protein

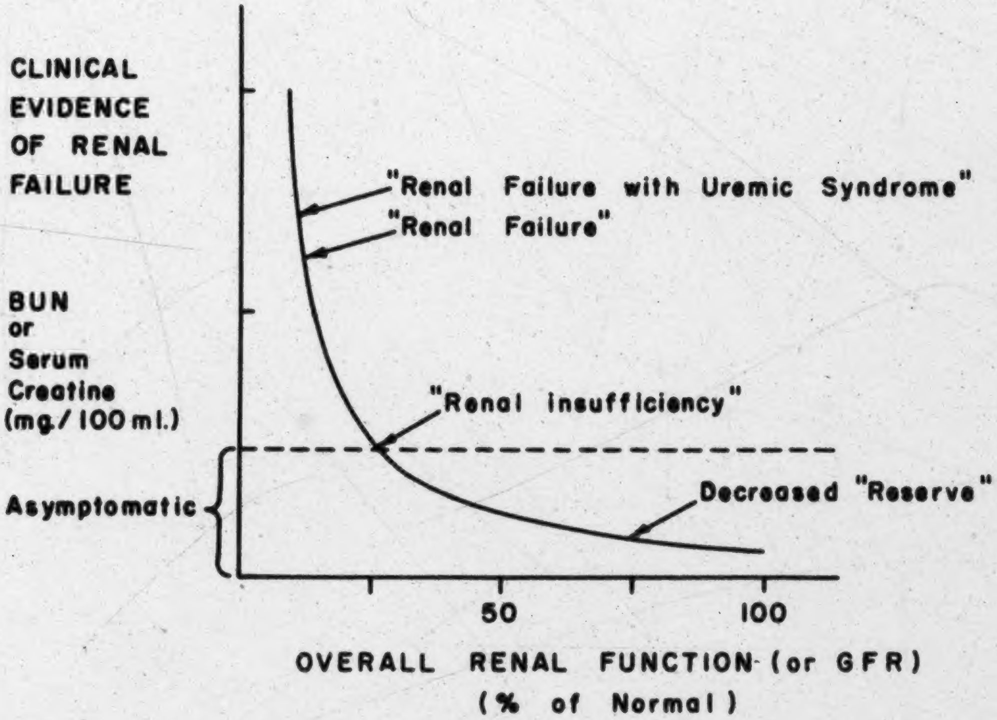


Fig. 6. The relationship of clinical manifestations (BUN/serum creatine) to renal function. (Modified from a diagram in F.H. Epstein. The treatment of reversible uremia, Yale J. Biol. Med. 27:53, 1954. The Yale Journal of Biology and Medicine, Inc.).

catabolism, an increase of the BUN above the normal range occurs when the GFR is reduced to 30 to 40% of normal (figure 6). This degree of depression of renal function at which the BUN rises may be markedly affected if the rate of protein metabolism changes. If catabolism is rapid as in trauma the BUN may rise above normal limits even when the GFR is diminished by only 50% or less. Conversely, when protein catabolism is slow, as in protein starvation and chronic disease, the rise of BUN into the abnormal range may be delayed until the GFR is reduced to 20% of normal. Consequently the rate of protein catabolism must be considered in interpreting the BUN.

It has been known for a long time that urea, creatinine, guanidine, and methyl guanidine and its derivatives are potentially toxic if they accumulate in uremia. Nephrectomized dogs, when maintained by peritoneal dialysis with a high urea bath, produce features of uremia (27). Foster in 1915 isolated from uremic blood an organic base which when injected into the guinea pig caused toxic symptoms, for example, rapid breathing, muscular twisting, convulsions and death (48). Harrison et al. (49) isolated guanidine or guanidine like derivatives, and Olson studied creatinine, potassium, nephrolysins and phenols in uremic patients to correlate them with the severity of the disease (50). It has been shown that upon injection of guanidine, methyl guanidine (which accounts for all the mono substituted guanidines that accumulate in chronic uremia) produces twisting, convulsions, coma, hemorrhages, gastroenteritis, erythrocyte hemolysis, pruritis, hemolytic anemia, thrombocytopenia, anorexia, vomiting, diarrhea, gastro-intestinal ulcers, peripheral neuropathy, twitching, ataxia, muscular hypertoxicity and other symptoms in dogs (20,51). Siminhoff et al., and Kramer reported accumulation in uremic blood of urea, methyl urea, aliphatic and aromatic amines, creatinine, creatine, uric acid, certain aminoacids, polypeptides, indican, hippuric acid, conjugates of phenols, phenolic and indolic acids and their conjugates; organic acids of the TCA cycle, guanidine bases, acetoin, 2,3-butylene glycol (52,53) and glucuronic acid and indican and indoles (by intestinal bacterial action) (54). High concentrations of guanidinosuccinic acid in the urine of uremic patients was reported (55,56). Cohen et al. speculated that nitrogen retention led to alterations of the pathway of ammonia detoxication and urea synthesis and repression of normal enzyme activity, and either the activation of dormant enzymes or the appearance of new enzymes (57). Stein et al. also reported increased serum and cerebrospinal fluid concentrations of GSA and confirmed the high urinary excretion in chronic renal failure (58). Giovanetti et al. in 1968 reported the presence of high concentrations of methyl guanidine, a constitutent of creatine (methylguanido acetic acid) and a derivative of creatinine, in uremic plasma (59).

The alternate metabolic pathway for urea synthesis (as described under "urea cycle") in chronic renal failure proposed by Cohen *et al*. is consistent with an increase in methyl guanidine concentration. Giovanetti *et al*. demonstrated the high toxicity of methyl guanidine and its catabolic effect in experimental dogs (60). It was shown that antibiotics, by reducing intestinal urea splitting microorganisms and the formation of ammonia, can limit synthesis of non-essential aminoacids and the amount of protein synthesis in uremics on very low protein diets (61).

2. Urea-biosynthesis

Urea represents the nitrogen equilibrium between the dietary intake of nitrogen and the sum of the daily renal excretion of nitrogen in the form of diverse compounds (for example, creatinine, uric acid) and ammonia. Formation and excretion of urea is the body's "levelling device". That is, a positive nitrogen balance leads to diminution of urea excretion and a negative nitrogen balance is due to excessive nitrogen excretion as urea at the expense of body protein.

The large amounts of ammonia formed by deamination of aminoacids are highly toxic. The animal body detoxifies it rapidly in the liver before release into the systemic circulation by conversion to urea through the urea cycle. Ornithine and citrulline were known to increase the rate of urea production. Arginine was found to be an intermediate product of the reaction. The liver enzyme arginase hydrolyzes arginine to ornithine and urea. Krebs and Henseliet proposed the cyclic mechanism for urea synthesis through ornithine, citrulline, arginine, ammonia and CO_2. Thus urea synthesis is a complex process which involves primary fixation of CO_2 and NH_3, and the formation of citrulline and its conversion to arginine (62-64).

The first stage in the urea synthesis is the formation of carbamyl phosphate formed by the reaction of NH_4^+ and HCO_3^- in the presence of ATP and Mg^{++}, and catalyzed by carbamyl phosphate synthetase (63). Carbamyl phosphate converts ornithine to citrulline, mediated by liver enzyme ornithine transcarbamylase. Arginine synthesis stems from citrulline which condenses with aspartic acid to form arginosuccinic acid under the influence of argininosuccinate synthetase. The product splits into arginine and fumarate as a result of cleavage by the enzyme arginine synthetase. Arginine is then hydrolyzed to ornithine and urea by arginase; this reaction, which results in the formation of urea, occurs only in liver. Of the two N atoms of urea, one is derived from ammonia through carbamyl phosphate and other from aspartic acid through argininosuccinic acid (see figure 7).

The process is endergonic, requiring in the order of 10 KCal/mole. Coupling of the transamination reaction and the reversible oxidative deamination of glutamic acid

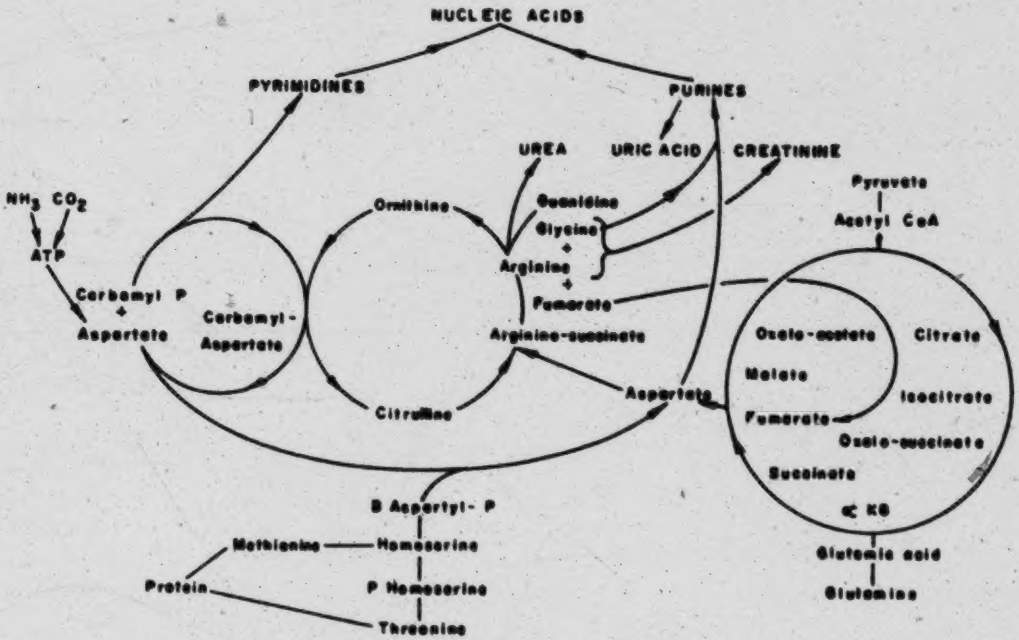
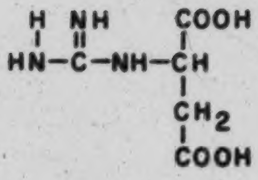

NUCLEIC ACIDS
PYRIMIDINES
PURINES
UREA
URIC ACID
CREATININE
NH_3 CO_2
ATP
Carbamyl P
+
Aspartate
Carbamyl-
Aspartate
Ornithine
Guanidine
Glycine
+
Arginine
+
Fumarate
Arginine-succinate
Citrulline
Aspartate
Pyruvate
Acetyl CoA
Oxalo-acetate
Citrate
Malate
Isocitrate
Fumarate
Oxalo-succinate
Succinate
α KG
Glutamic acid
Glutamine
B Aspartyl- P
Methionine
Homoserine
Protein
P Homoserine
Threonine

Fig. 7. Metabolic interrelationships of certain nitrogenous substances, the end products of which become elevated in nephron failure. (Nephron Failure by J.B. Dossetor and M.H. Gault, Pub. Charles C. Thomas, 1971).

to α-keto glutarate and ammonia is the mechanism for removal of the amino group from amino acids as NH_3, and also "fixation" of NH_3 into aminoacids as - NH_2 groups (65).

Aspartic acid contributes its nitrogen atom to urea via argininosuccinate, an intermediate in arginine formation (as shown in figure 7). Aspartate formation from glutamate via transamination with oxaloacetate provides a mechanism for channelling the amino group from amino acids into urea (see figure 8). Recently, the isolation of guanidino succinic acid (GSA) from uremic blood supports the evidence that feed back inhibition occurs in uremia. Normally, GSA is found only in uremia, but is not detectable in normal blood. In uremia, the GSA present in the blood is built up of creatinine, creatine and guanidinoacetic acid GAA) (57,66). GAA is synthesized in feedback inhibition of amidinotransferase which converts glycine and arginine to ornithine and guanidinoacetic acid (see figure 8), that is, by the alternative pathway of urea synthesis as proposed by Cohen et al. (66). If the normal metabolic pathway is inhibited by retained nitrogen, and aspartate-arginine amidinotransferase enzyme is present, then utilized arginine could be shunted into the synthesis of GSA.

Cohen et al. also proposed a possible scheme for reutilization of urea for the biosynthesis of non-essential amino acids (see figure 9) (66) in which GSA originates as an intermediate.


H NH COOH
HN–C–NH–CH
CH2
COOH

Guanidinosuccinic acid

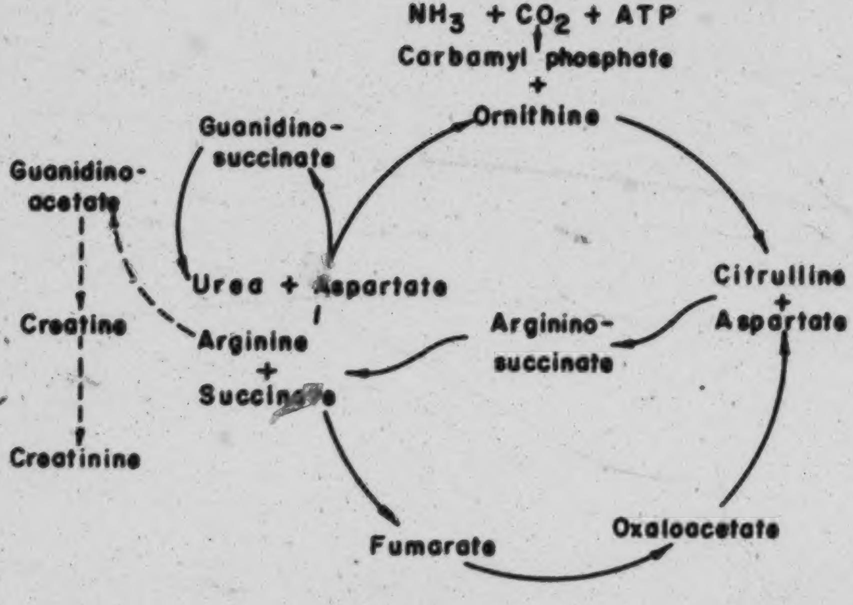

NH3 + CO2 + ATP
Carbamyl phosphate
+
Ornithine
Guanidino-
succinate
Guanidino-
acetate
Urea + Aspartate
Citrulline
+
Aspartate
Creatine
Arginine
+
Succinate
Arginino-
succinate
Creatinine
Fumarate
Oxaloacetate

Fig. 8. Possible alternate route for synthesis of urea. This is predicated on the existence of an aspartate-arginine amidino-transferase, an enzyme which would be analogous to that resulting in the transamidation of glycine (Cohen, B.D. et al., Amer. J. Clin. Nutr. 21, 107 (1968).

Each step of the urea cycle (figure 8) is reversible up to but not including the breakdown of arginine to form urea and ornithine. Reutilization of urea, in essence, implies reversibility of this latter step (figure 9).

E. The Uremic Syndrome

Symptoms of the uremic syndrome involve many body systems (67,3,20), including the gastro intestinal system, the cardiovascular system, the blood, the nervous system, the respiratory system and the skeleton.

1. The Gastro-intestinal Symptoms

These symptoms are characterized by anorexia, nausea, vomitting, hiccups, lethargy, somnolence and excessive thirst. Upper gastro-intestinal manifestations include a dark tongue, uremic breath odour, a taste of ammonia and ulceration anywhere from the mouth to the anus. "Uremic frost" on lips, a lead taste in the mouth, and discoloration of the skin (urochrome or urinary pigment, a sallow coloration) may be present.

2. Cardiovascular Symptoms

These symptoms are manifest as hypertension, edema, and pulmonary congestion.

(a) Hypertension

More than 80% of uremic cases develop hypertension. The causes are of two fold:

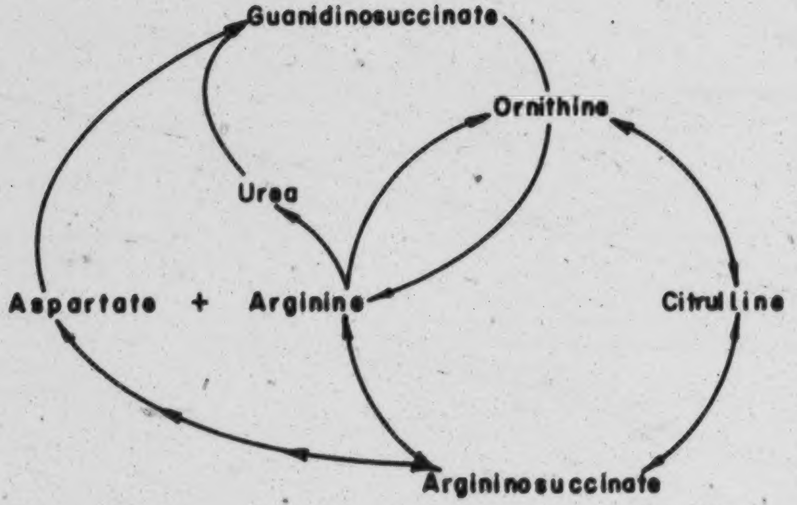

Guanidinosuccinate
Ornithine
Urea
Aspartate + Arginine
Citrulline
Argininosuccinate

Fig. 9. Possible scheme for reutilization of urea. Urea condenses with aspartate to form guanidinosuccinate, which in the presence of ornithine restores arginine. Thus the breakdown of arginine is reversible and permits the entrance of urea into a number of protein anabolic pathways. (Cohen, B.D. et al., Am. J. Clin. Nutr. 21, 407 (1968)).

i. the increased secretion of renin by the juxtaglomurular apparatus (JGA). The JGA consists of a portion of the distal tubules which lie close to the afferent and efferent arteriols and the glomerulus of the same nephron. The distal tubular cells (more columnar), called macula densa, the J-G cells, and the cells between the distal tubule and the glomerulus (called Lacis cells) form the entire JGA (see figure 10). These cells of JGA are sensing and also secretory organs, and are sensitive to a change of pressure within the afferent arteriole. Recognizing a decrease in perfusion "pressure", they respond by secreting renin. Renin, a proteolytic enzyme in the plasma acts on an α-2-globulin to produce angiotension I, a decapeptide. Angiotension I is converted by enzyme action to angiotension II, an octapeptide (a hypertensive). Angiotension II, a hypertensive, is also a potent stimulus to aldosterone secretion by the adrenal gland. Thus angiotension II causes arteriolar constriction (hypertension), and an increase in blood pressure.

ii. there is an expansion of effective extra cellular fluid volume and blood volume (EECFV) which decrease renal secretion of sodium. A decrease in EECFV is noted by J-G cells, which secrete renin, followed by the formation of angiotension. Angiotension then stimulates aldosterone secretion which stimulates reabsorption of sodium and expansion of EECFV, (see figure 11). The

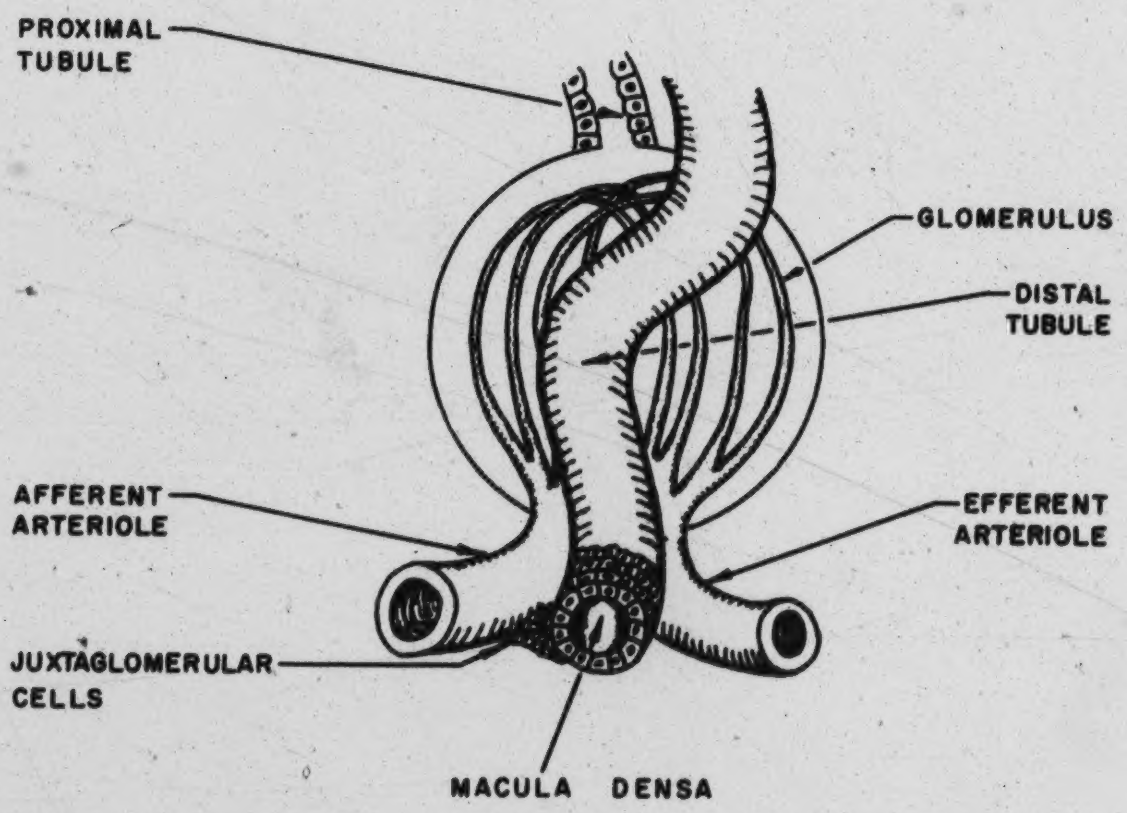

PROXIMAL TUBULE
GLOMERULUS
DISTAL TUBULE
AFFERENT ARTERIOLE
EFFERENT ARTERIOLE
JUXTAGLOMERULAR CELLS
MACULA DENSA

Fig. 10. The juxtaglomerular apparatus.

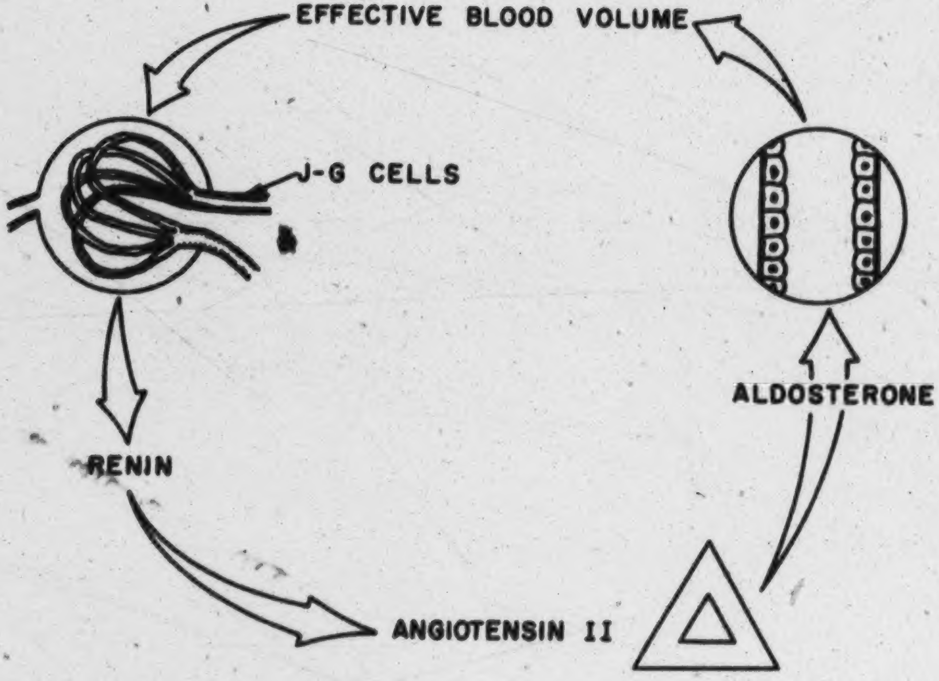

EFFECTIVE BLOOD VOLUME
J-G CELLS
RENIN
ALDOSTERONE
ANGIOTENSIN II

Fig. 11. The renin-angiotensin-aldosterone system.

increased blood volume causes an increased cardiac output which provides too much blood flow through the tissues.

(b) Edema

Edema is caused by hypertension and impaired sodium and water excretion; it is characterized by pulmonary edema and the "uremic lung" (the lung becomes infiltrated with macrophages and the fibrous septa thickens).

(c) Blood Chemistry

Anemia is associated with chronic renal failure. The causes may be non-uremic, due to peptic ulcer and folate deficiency from malnutrition (a common feature in uremia); uremic anemia may be due to the accumulation of metabolites which shorten the life span of red blood cells (120 days to 90 days); they can be corrected by frequent dialysis, though dialysis is also associated with an increment tendency towards anemia. In normocytic and normochromic anemia, a deficiency of erythropoietin incapacitates the bone marrow to response to small amounts of bleeding and hemolysis (see figure 12) (68). Erythropoietin stimulates erythropoiesis or production of red blood cells in the bone marrow. The kidney, being the major source of erythropoietin, fails to release enough renal erythropoietic factors in uremia.

The hemoglobulin count falls below 7 g/100 ml and other side effects such as dyspnea, angina, injection systolic murmurs, and elevated venous pressure are common.

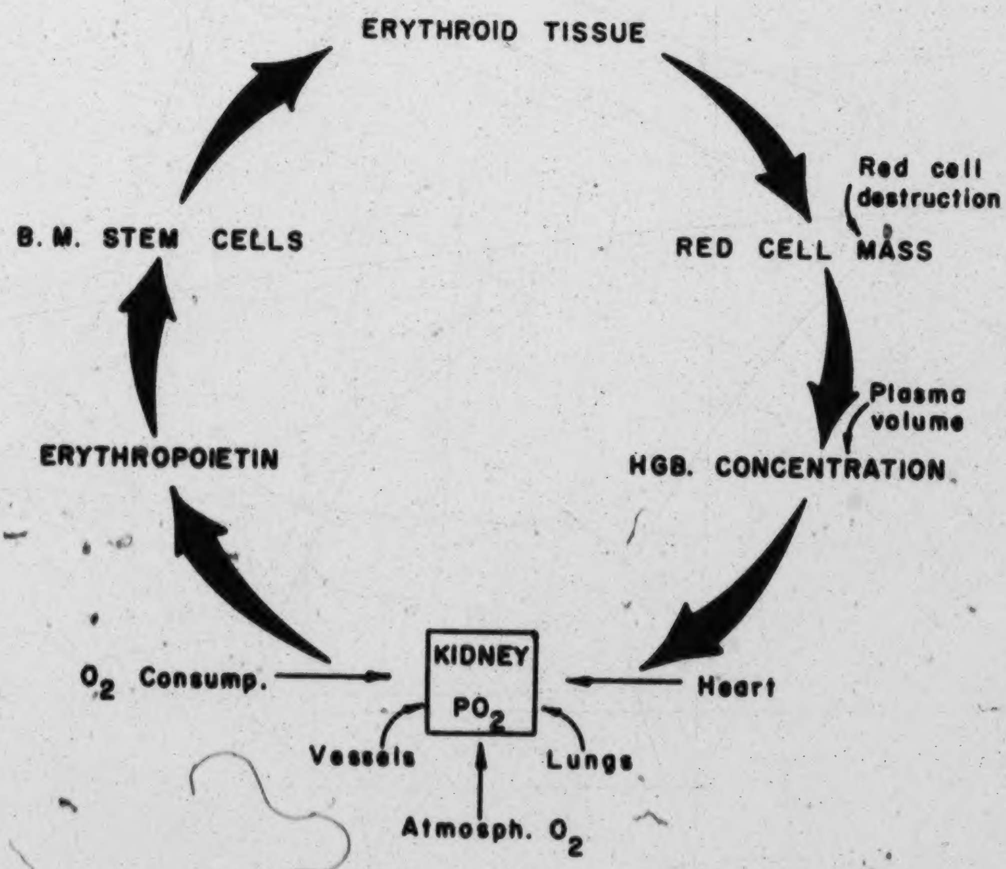

ERYTHROID TISSUE
Red cell destruction
RED CELL MASS
Plasma volume
HGB. CONCENTRATION
Heart
KIDNEY
PO_2
Lungs
Atmosph. O_2
Vessels
O_2 Consump.
ERYTHROPOIETIN
B. M. STEM CELLS

Fig. 12. Erythropoietin and red blood cell formation. (The Kidney, Vol. 1, No. 4, March, 1968).

3. The Nervous and Respiratory System

Twitching, convulsions, delirium and coma preceded by Grand mal seizures (epilepsis) are common nervous symptoms in uremia. Convulsions may be due to hyponatremia, hypertensive encephalopathy, hypocalcemic tetany or the uremic state.

The respiratory system symptoms include difficulty in breathing (dyspnea), chyne-stokes respiration (the death rattle) or Kussmaul breathing (deep signing respiration) due to the attempts made by the body to correct metabolic acidosis.

4. Bone Lesions (Renal Osteodystrophy)

(a) Osteomalacia (inadequate mineralization of bone) is due to Vitamin D resistance and the use of skeletal calcium carbonate to buffer acidosis. Examples are rickets in children and bone pain in adults. The treatment used is Vitamin D (50,000 to 150,000 units daily) plus 4 to 12 g Ca-gluconate or 1,25-dihydroxycholecalciferol.

(b) Ostietis fibrosa or bone cyst, consists of osteoclastic absorption of bone and its replacement by fibrous tissue. The treatment is to reduce parathyroidism by raising serum Ca with Vitamin D and Ca-gluconate.

(c) Osteosclerosis or metastatic calcification occurs due to an increase of bone density in the vertebra, face and skull bones. Metastatic calcification of joint, synovia, tendon sheath, eyes, blood vessels, and skin may be due to deposition of calcium salts.

Other symptoms exhibited in uremia are albuminurea, and hypoalbuminemia, lipidurea, hyperlipemia, diabetes, amyloidosis, multiple myeloma, collagen disease, renal vein thrombosis, nephrotoxins, and infections.

F. The Impact of Renal Failure on Metabolism

Metabolic defects due to uremia are known to occur in the utilization of carbohydrate, fat, protein, enzymes, production of energy, membrane transport and electrolytes. The changes thus incurred are cellular or subcellular, through the metabolic pathways, the repression of enzymes, and the regulation of genes. Most of these metabolites, secondary to uremia are known to be dialyzable with a molecular weight ranging from 5,000 to 10,000. Any defect may be associated with abnormalities of the nervous systems (central and peripheral), hemopoiesis, epithelium (especially the gastro-intestinal tract, vascular endothelium) and serous membranes. Abnormalities in the body fluid or concentrations of Na^+, K^+, Ca^{++}, Mg^{++}, Fe^{+++}, and H^+ ions, abnormal metabolism of proteins, purine, and pyrimidine and deranged synthesis or function of nucleic acids, nucleotides, or nucleoproteins, and extracellular concentration of their nitrogenous end products may occur.

1. Fluids and Electrolytes

A person with a 70 Kg body weight has approximately 42 liters of body water comprising 28 liters of intracellular and 14 liters of extracellular fluids. Plasma comprises 4% of the body water (2.8 liters).

In extracellular fluids, Na^+ is predominantly present; the 155 mEq/ℓ of cation consists of Na^+, K^+, Ca^{++}, and Mg^{++}, and the 155 mEq/ℓ of anion consists of HCO_3^-, Cl^-, $HPO_4^=$, $SO_4^=$, organic acids, and protein. The net result is a neutral osmolality of extracellular fluid. Any change in osmolality reflects changes in sodium, the predominant cation.

In the normal steady state, the rate of endogenous acid production is more or less equal to the rate at which the tubules secrete protons and add new bicarbonate to the renal venous plasma (69). The origin of tubular secretion of protons might be from carbonic acid derived within the cells by the hydration of CO_2 (70), by carbonic anhydrase or by removal of hydroxyl ions from within cells by carbonic anhydrase thus preventing the cells from becoming too alkaline (71,4). Regardless of the source of secreted protons, it is agreed that the secretion of protons is coupled to the reabsorption of sodium from the tubular fluid (5). The secreted protons are almost entirely buffered by phosphate and ammonia in the tubular fluids and thus the net excretion of acid in the urine can be measured by the sum of NH_4^+ and titratable acid PO_4, less any bicarbonate in the urine which escaped reabsorption from the glomerular filtrate. The kidney maintains the electrolyte balance by excreting anions (derived from fixed metabolic acids), the mechanism of which is operationally separate from tubular acid secretion and which primarily depends on volume of glomerular filtrate. For example, in tubular acidosis (a disorder of tubular capacity of excretion) a slight reduction of the GFR is observed, with no retention of the anions ($PO_4^=$ and $SO_4^=$) of metabolic acids. Sustained acidosis slowly dissolves mineral salts in the skeleton to retain acidity without a progressive fall in plasma bicarbonate. The release of alkaline calcium salts from bone might be an important mechanism for neutralizing excess acid, as the kidney in chronic renal failure fails to retain normal plasma bicarbonate. This leads to extreme degrees of demineralization of bone. Treatment of such patients with alkali may be beneficial to help prevent utilization of the skeleton as a buffer reservoir (72,73,74).

In metabolic acidosis, the urinary pH is below 5.0 units. The reduction in acid excretion results primarily from reduced functional renal mass but ammonia excretion per nephron increases adaptively in the residual nephrons (75). Acidosis directly enhances ammonia release in the kidney by stimulating glucose production at some point in the gluconeogenesis pathway beyond oxaloacetate (76).

In balance studies, Schwartz et al. (77) reported that in some patients with chronic renal failure and acidosis there was renal tubular "bicarbonate wasting" when their plasma bicarbonate concentration was raised to normal, or nearly normal.

Under normal conditions, an ordinary diet results in an acid "excess" of approximately 50 mEg of H^+ ions daily. Aside from the buffering effects of body cells there will be buffering by the major plasma buffer systems (carbonic acid-bicarbonate: H_2CO_3 - HCO_3^-). The relation of $H^+ + HCO_3^- \longleftrightarrow H_2CO_3 \longleftrightarrow H_2O + CO_2$ is commonly written by the Henderson-Hasselbach equation:

$$pH = pK + \log \frac{(HCO_3^-)}{H_2CO_3},$$

since most of the carbonic acid is in the gas form, pH is the function of the ratio: (see figure 13).

$$\frac{(HCO_3^-)}{pCO_2}$$

If, H^+ is added to the system, the equation shifts to the right, lowering bicarbonate and increasing CO_2 production. CO_2 is then carried to the lungs and expired. This minimizes the change in ratio of $\frac{HCO_3^-}{pCO_2}$ or pH, and is called "compensation". In spite of respiratory compensation, the organism has excess H^+ ions and the equation has moved to the right, thus lowering serum bicarbonate. Therefore, the kidney has to perform this dual role of:

(a) Eliminating the Excess Hydrogen Ions.

(b) Regenerating Bicarbonate by the Following steps:

i. complete reabsorption of the filtered bicarbonate in the proximal tubule to prevent further reduction of serum bicarbonate by converting HCO_3^- to H_2O and CO_2 by H^+ secretion in the lumen (indirect process). The CO_2 then diffuses back into the cell, shifting the reaction towards formation of H_2CO_3 and HCO_3^-. The intracellularly generated HCO_3^- is delivered to the extracellular fluid with sodium absorbed in exchange for secreted H^+ ions.

ii. although the filtered HCO_3^- is converted to H_2O and CO_2, H^+ secretion continues in the distal nephron

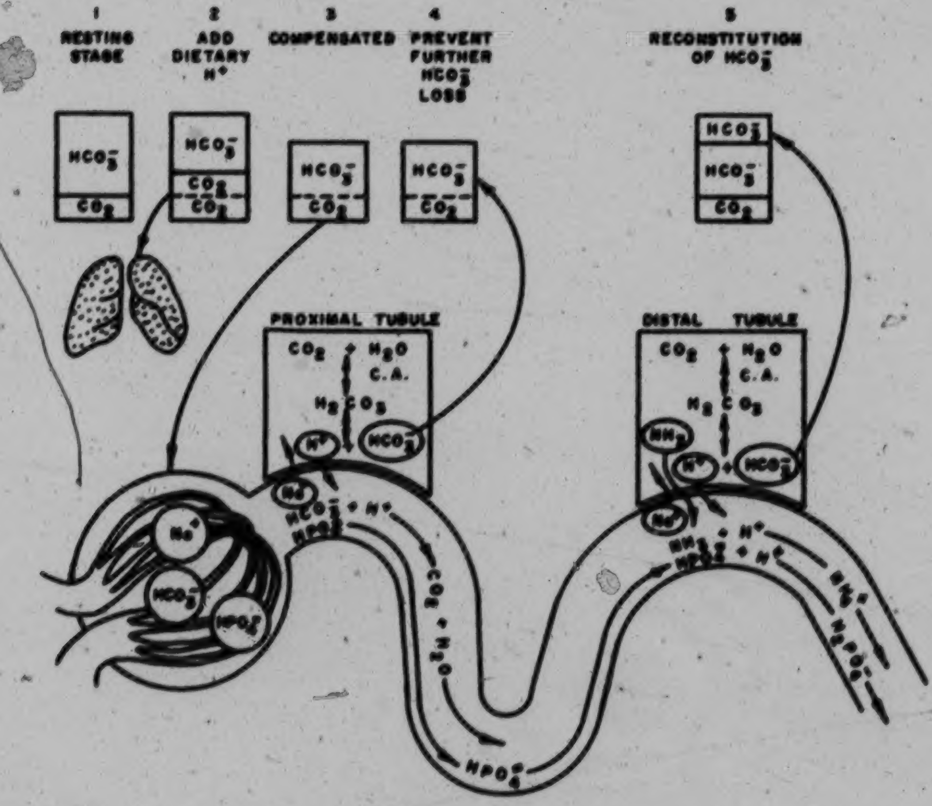

1
RESTING STAGE
2
ADD DIETARY H^+
3
COMPENSATED
4
PREVENT FURTHER HCO_3^- LOSS
5
RECONSTITUTION OF HCO_3^-
HCO_3^-
CO_2
PROXIMAL TUBULE
DISTAL TUBULE
$CO_2 + H_2O$
C.A.
H_2CO_3
H^+
NH_3
Na^+
$HPO_4^=$
$CO_2 + H_2O$
$H_2PO_4^-$

Fig. 13. Renal handling of the daily dietary acid (hydrogen ion) load. (Modern Treatment, Vol. 5, July, 1968, Dr. Bricker and Hoeber Medical Division, Harper & Row, Publishers, Inc.).

where it is buffered by filtered buffers. For example, $HPO_4^{=}$ (titrable acid) forms $H_2PO_4^-$ or ammonia is secreted in the distal tubular cell in response to acidosis and is excreted as the ammonium ion. Thus, elimination of H^+ without lowering the pH and damaging tissues occurs. The hydrogen that is secreted is derived from cell H_2CO_3, leaving HCO_3^- behind in equimolar amounts. This bicarbonate, together with sodium reabsorbed in exchange for secreted hydrogens, moves into the ECF to reconstitute the serum bicarbonate (see figure 14).

In clinical disturbances of acid-base equilibrium the numerator (HCO_3^-) is largely regulated by the renal mechanism of H^+ secretion. Bicarbonate regeneration, (the denominator, pCO_2), is primarily regulated by pulmonary mechanism. A change in the numerator or denominator is followed by a unidirectional change in the other. This serves to defend the pH and the phenomenon is called compensation.

In acidosis, HCO_3^- decreases from 24 mEq/ℓ to 15 mEq/ℓ, and pCO_2 decreases from 40 mm Hg to 25 mm Hg; in compensation, the normal ratio of HCO_3^-/H_2CO_3 is restored to 20:1 as a result of lowered H_2CO_3 (from 1.2 mEq/ℓ to 0.75 mEq/ℓ). The normal pH (7.4) in acidosis is 7.2; in compensation it is partially restored, to 7.3.

In alkalosis, HCO_3^- increases from 24 mEq/ℓ to 38 mEq/ℓ, and pCO_2 increases to 45 mm Hg from 40 mm Hg,

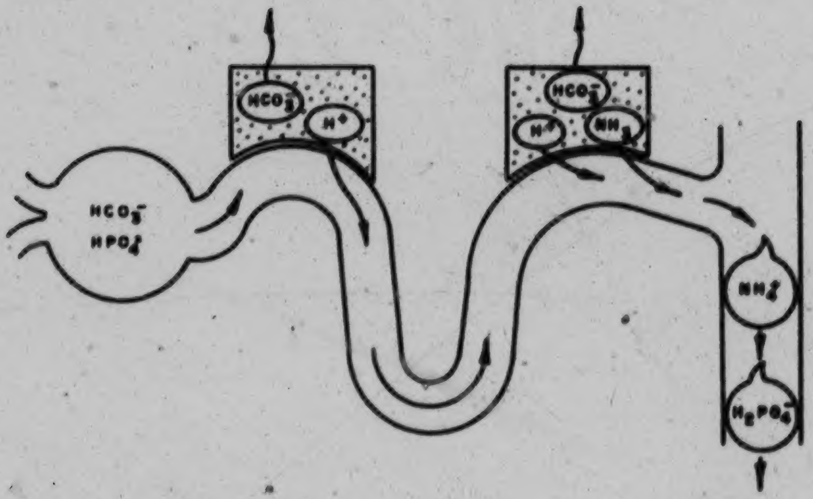

HCO3-
HPO4=
HCO3
H+
HCO3-
H+
NH3
NH4+
H2PO4-

Fig. 14. Oversimplified schema of the renal regulation of acid-base. (Modern Treatment, Vol. 5, July, 1968).

in compensation, the HCO_3^-/H_2CO_3 ratio is partially restored (from 31.6:1 to 25.6:1). The pH is reduced to 7.55, from an uncompensated level of 7.6. Thus if the numerator (HCO_3^-) decreases it is metabolic acidosis, and if the numerator increases it is metabolic alkalosis (hypoventilation). If the denominator (H_2CO_3) increases it is respiratory acidosis, but if the denominator decreases it is respiratory alkalosis (hyperventilation). In uremic acidosis there is K^+ depletion (hypokalemia) causing periodic paralysis, HCO_3^- decrease (hyperchlorimic less than 24 mEq/ℓ), Ca^{++} decrease (nephrocalcinosis, hypocalcemia), hyperparathyroidism, amino aciduria, glucosuria, and uricosuria.

2. Calcium and Phosphate Homeostasis in CRF

The active physiological fraction is that fraction of Ca which is ionized. This active species triggers a feedback mechanism for the secretion of parathyroid hormone and calcitonin by the C-cells of the thyroid. The mean Ca concentration in plasma is 2.50 mM per liter, of which 1.42 mM are diffusible (1.27 mM ionized and 0.15 mM conjugated with bicarbonate, citrate, phosphate and other anions) and the rest (1.08 mM) are non-diffusible, being protein bound. Of the bound portion, 0.86 mM are bound to albumin and 0.22 mM are bound to globulin) (1,78).

In the absorption of Ca from the gut the dominant factor is Vitamin D (52) and its metabolites (25-hydroxy-cholicalciferol formed in the liver) and (1,25-dihydroxy-cholecalciferol formed in the kidney) (79). Either vitamin D or one of its metabolites, enhances Ca absorption through the synthesis of a specific calcium binding transport protein in mucosal cells of the G.I.T.

Nordin et al. (80) postulated that the kidney is the most important organ in Ca homeostasis, and suggested that it is the action of parathyroid hormone on the tubular reabsorption in Ca which finally determines plasma Ca concentration. They considered that the acute hypocalcemic action of calcitonin in man was largely responsible for a reduction in the tubular reabsorption of Ca. They hypothesized that the action of PTH on bone resorption represents a reserve mechanism to mobilize bone Ca if Ca intake or absorption falls below a critical level.

The major portion of plasma phosphate is in an inorganic form as orthophosphoric acid ions (81) with a small non-ultrafiltrable, protein bound fraction (82). The G.I.T. absorption of phosphate is controlled by vitamin D and the transport is mediated by two routes - one independent of Ca is mediated by diffusion and the other depending on an active transport mechanism is linked to Ca (1:1 molar ratio) (83). The kidneys are probably the most important organs in plasma phosphate homeostasis for as Fourman et al. stated, "In a steady state the kidneys excrete what the gut absorbs" (84). Glucose, vitamin D, and parathyroid hormone influence the tubular reabsorption of phosphate (6,85,86). Meyer et al. reported that hyperphosphatemia stimulates the secretion of PTH only indirectly by inducing hypocalcemia; vitamin D is effective through its effect on the G.I.T. transport. Renal tubular handling is one of the major factors in plasma phosphate homeostasis.

In CRF, the plasma phosphate concentration rises. Plasma inorganic phosphate is derived from the diet and may be liberated from organic compounds such as phospholipids and nucleoproteins in catabolic states. The increase of plasma phosphate concentration could be due to a reduction of functional renal mass, though the average rate of phosphate excretion per residual nephron increases as the nephron population is reduced. In this mechanism PTH is the important factor (87). The disturbance in Ca and P homeostasis commonly takes the form of hypocalcemia and hyperphosphatemia in CRF.

3. Role of Vitamin D and PTH in Ca and P Homeostasis, and Their Resistance in Uremia

Vitamin D was thought to act directly via RNA in the induction of the specific protein which transports calcium in the intestinal mucosal cells. The stage at which the vitamin affects RNA synthesis has not been localized (78).

It is now proposed that vitamin D is first metabolized to a more polar compound which through RNA induction accounts for the characteristic actions of the vitamin D (88); De Luca suggested that the biologically active metabolite is 25-hydroxycholecalciferol (in liver) and 21,25 DHCC and 1,25DHCC (in intestine, kidney and bone) (79). Lawson _et al_. have suggested that an as yet unidentified more polar metabolite is involved. In 1968, Avioli (89) demonstrated that the insensitivity to vitamin D in CRF is due to an acquired defect in the metabolism and excretion of the vitamin, and that alterations in enzyme systems regulating vitamin D metabolism are probably inhibited by retained metabolites. This in turn leads to a decrease in the specific calcium binding protein content of the intestinal mucosa and the plasma concentration of 25-hydroxycholecalciferol.

PTH exerts both its renal and skeletal action through cyclic 3',5'-AMP (90) and through induced synthesis and release of the lysosomal enzymes of bone cells (91). Murad _et al_. suggested that calcitonin enhances the formation of cyclic 3',5'-AMP in a way identical to PTH, and an increase in the intracellular concentration of cyclic 3',5'-AMP activates a cellular enzyme in the kidney initiating a series of reactions leading to elimination of phosphate from the cell (92).

The lack of response to PTH in the vitamin D deficient state is proposed to be due to a local critical lack of Ca at the site of hormonal action (93,94), as Ca is needed for cyclic 3',5'-AMP to exert some of its action (95). Therefore, the acquired resistance to PTH in hypocalcemic chronic renal failure is due to a critical lack of Ca to mediate some of the actions of cyclic-3',5'-AMP. The CRF thus causes vitamin D resistance with consequent hypocalcemia leading to osteomalacia and PTH stimulation. PTH resistance also increased PTH secretion and causes ostitis fibrosa and osteoclerosis.

4. Sodium and Potassium Homeostasis in CRF

CRF patients have an impaired ability to conserve sodium due to the increased osmotic load per residual nephron, as the kidney is directly implicated in disorders of sodium and water balance. Salt wastage, common in CRF, is due to the inability of the surviving nephrons to lower sodium concentrations of tubular fluid below a relatively high fixed value; this in turn is due to an increased osmotic load per nephron. The latter is responsible for the tubules' impaired ability to concentrate urine because of the diminished response of the tubules to ADH.

In the early stages of CRF, the impaired ability to conserve sodium and the associated hyponatremia dictates the necessity for administration of sodium to prevent depletion. In the latter phase, the situation is reversed and a different pattern of sodium homeostasis, as manifested by uncontrollable hypertension, prevails (96).

Control of hypertension at this end stage can be achieved by intermittent hemodialysis and sodium restriction between dialyses. By dialysis, extracellular fluid volume and exchangeable sodium can be reduced to normal or near normal levels (97).

5. The Role of Hormones in Sodium and Water Homeostasis

Normally, aldosterone and ADH regulate the sodium and water balance. No dominant disturbances in secretion of aldosterone in non-edematous patients have been seen (97), but the tubules show a diminished response to adequate circulating levels of ADH (98). The failure of ADH responses is mainly due to osmotic diuresis occurring in individual surviving nephrons (99).

In CRF, there is a reduction of cortisol clearance (100) and there is an inverse relationship between endogenous creatinine clearance and endogenous conjugated plasma hydroxycorticosteroid (17-OHCS) levels. After cortisol infusion, the rate of clearance of free plasma 17-OHCS is reduced and levels of the conjugated steroids increased further. This defect in clearance correlated with the degree of functional renal impairment.

In females, fertility is depressed and secondary ammenorrhoea or menorrhagia occurs in CRF (101). In males, fertility is reduced to a lesser extent than in females and normal sexual activity continues unimpaired (102).

6. Potassium

Potassium intoxication in ARF is a potentially lethal factor, but in CRF hyperkalemia occurs only in terminal stages. Potassium is released from the cells after catabolism of protein and acidosis. In acidosis the increased H^+ ion concentration in the extracellular fluid is exchanged at the expense of intracellular potassium concentration (1). The infrequent hyperkalemia in chronic renal failure is usually credited to the ability of the diseased kidney to maintain the normal level of urinary potassium excretion by tubular secretion.

7. Magnesium

Magnesium activates some of the enzymes which split and transfer phosphate groups, namely phosphatases and enzymes involving ATP reactions. ATP is required in muscle contraction, in the synthesis of protein, nucleic acid, fat and co-enzymes, in utilization of glucose, and in oxidative phosphorylation. All of these diverse functions are activated by magnesium (21).

Hypermagnesemia as a feature of chronic renal failure has been reported by many workers (103-105). Because of the role of magnesium in cellular metabolism, hypermagnesemia has been implicated as a cause of neurological manifestations seen in CRF (106,107); normal values of serum magnesium have also been reported (108). Clarkson _et al_. (109) studiedthe effect of a high Ca intake on magnesium in both normal and uremic patients and observed that a diminuation in magnesium absorption and an equivalent fall in urinary excretion was associated with a fall in plasma magnesium concentration, due to the inhibition of parathyroid hormone secretion secondary to the rise in Ca absorption and plasma Ca concentration.

8. Iron and Vitamin Homeostasis in Uremia

In CRF, the metabolism of iron and hemoglobin is known to be altered and anemia of both normochromic and normocytic types reflecting alterations in cellular metabolism prevail and cannot be corrected by dialysis. Evidence of bone marrow depression was presented by a decrease in the rate and amount of radioactive iron required for hemoglobin synthesis (110,111). This defect in iron utilization was attributed to an inadequate production of erythropoietin. Riessman (112) was first to report the presence of this humoral factor which mediates the stimulus to erythropoiesis; its renal origin was later shown by other workers (113-116). The dominant factor in the etiological mechanism of anemia in CRF is deficient erythropoiesis consequent to a diminution in erythropoietin production (117,118); other contributing factors may be hemolysis, hemorrhagic diathesis, increased red cell destruction and bleeding (110,111,119,120,121).

9. The Role of Other Vitamins

In most uremic serum, increased concentrations of Vitamin B_{12} and low concentrations of folic acid are observed (122,123). In CFR, biotin and nicotinic acid levels range with values from high to low. The anemia in CRF is, therefore, not megaloblastic as levels of folic acid and Vitamin B_{12} (which are involved in erythropoiesis and nucleic acid synthesis) are within normal limits. It is generally accepted that under physiological conditions, tissue hypoxia caused by anemia will lead to the appearance in plasma of an erythropoietic hormone, called erythropoietin which acts by differentiating bone marrow stem cells to early red cell precursors (124). The work of Naets (115) and Riessman (125) led to the hypothesis that red cell production is controlled by a feedback mechanism operating between the bone marrow and the kidney, mediated in one direction by red cell bound oxygen and in the opposite direction by erythropoietin as shown earlier in figure 12 (126).

Oral supplements of water soluble vitamins (which are likely to be removed during dialysis) are given to CRF patients maintained on intermittent dialysis in spite of high dietary intake (124).

10. Protein Metabolism

Hypoproteinemia and proteinuria are common characteristic features of renal failure (21). Hypoproteinemia in uremia is a consequence urinary loss of protein, low dietary intake (usually supplements are given to uremic patients) and defects in protein metabolism, the nature of which is not yet fully understood. Proteinuria may be caused by increased glomerular filtration of protein, decreased reabsorption of the filtered fraction, and protein loss from tubular cells.

Herndon et al. (56) in their balance study showed that uremic patients had increased endogenous nitrogen metabolism and required higher protein intake than normal subjects to maintain balance. Lacy (127) in his study of amino acid uptake by isolated uremic liver found that uremic rat liver showed increased amino acid uptake and urea production compared with control rat liver. Using an in vitro system with cell-free uremic rat liver preparations, McCormick et al. (128) in their labelled L-leucine incorporation study found that the rate of hepatic protein synthesis was increased in uremia which supports the work of Lacy (129). They concluded that, in relation to the negative nitrogen balance as seen in uremia, their finding indicated that either the rate of protein degradation was increased or the uremic liver synthesized abnormal or in-incomplete protein.

Most interesting is the alteration of the uremic serum protein fraction. In both chronic uremia and long term intermittent hemodialysis therapy serum protein and albumin levels range from high to low but the globulin fraction either remains normal or increases, showing abnormal immune response in all uremic patients (130). After homotransplantation in CRF, there was prolonged survival of the transplant with a much less intense local immune response (i.e. rejection) (131-134). Shafrir et al. (135,136) have shown that on administration of ^{14}C-glucose, protein synthesis is enhanced, and incorporation of ^{14}C into the aminonucleoside-induced nephrotic liver increased. An increase in the specific activity of albumin and α- and β-globulin and fibrinogen synthesis increased in quantity though they become less strongly labelled. They also found that incorporation of ^{14}C-glucose into nephrotic plasma protein and lipid exceeded that found in normal, but incorporation into tissue glycogen and skeletal muscle protein and depot fat was reduced. They suggested that conversion of glucose into lipids is consequent to the elaboration of lipoprotein peptides.

11. Amino Acids

The amino acid demands in the body require a few fundamental considerations:

(a) Dietary Intake of Protein,

(b) Absorption of Amino Acids in the Intestine,

(c) Transport Mechanisms of Aminoacids (in the Intestine at Least 3 Groups Exist),

(d) Metabolic Pathway (137,138).

Giordano et al. (139) studied labelling of 18 amino acids using labelled nitrogen of different sources, (e.g. amino acids, proteins, ammonia, urea, a molecular nitrogen) in uremic subjects on control diets containing normal, low protein or minimal quantities of essential amino acids. They found that of 18 amino acids only glutamic acid, alanine, aspartic acid, serine and glycine were labelled significantly more than others. When fed essential amino acids, a positive nitrogen balance was achieved with a gradual decrease of the urea level in the blood. The most interesting finding was that even when labelled molecular nitrogen was given subcutaneously in pyelonephritis there was significant incorporation into protein (139). Walser et al. (140) observed that more than 70% of body urea might be reutilized in uremia. It has been reported that recycled urea nitrogen is used in uremia for the synthesis of non-essential amino acids (141-143). This nitrogen is also available for the synthesis of some essential amino acids if their carbon skeletons are provided in the diet (144,145). Brown et al. (146) showed that protein restriction alone reduced the activity of the urea cycle enzymes, namely the arginine synthetase system enzymes, alanine amino transferase (AIT) and branched chain amino acid transaminase (BATase) but that uremia alone increases the activity of the arginine synthetase system enzymes and AIT; BATase was not altered. They also observed that in uremia, the arginine synthetase system enzyme activities (e.g. of which argininosuccinate synthetase is the rate limiting enzyme in the urea cycle) were increased in direct proportion to the blood urea concentration as a result of the substantial increase in ammonia which reached the liver from urea hydrolysis in the colon (147). Maier et al. (155) also found increased activity of alanine and aspartate amino transferase activity in the liver and higher plasma alanine in 12 hours starved and 48 hour nephrectomized rats. Muting et al. (148) reported that in uremia, serum alpha amino nitrogen increases significantly compared to normal subjects or patients with renal insufficiency. Particularly, concentrations of lysine, tyrosine, tryptophan, methionine, glycine and glutamine may be normal or 2 to 3 times greater than normal. This non-uniform increase of different amino acids in body fluids is due to the fact that there are separate amino acid transport pathways for different amino acid families, different affinities of amino acids for the same transport site, and possible effects upon the transport mechanism by some factor(s) in uremic serum (149). Patients with severe chronic uremia have a depression of plasma concentrations of both dietary essential amino acids (valine, isoleucine and tryptophan) and non-essential amino acids (tyrosine and alanine) though concentration of methionine, leucine and phenylalanine are normal. It is also speculated that depletion of specific intracellular amino acids may exist in uremia which can lead to impaired protein synthesis. The clinical implication is that if dietary care is not taken, dialysis may deplete further some of amino acids (157).

The occurrence of decreased gluconeogenesis prompted some workers to investigate the concentration of several glucogenic amino acids (e.g. aspartate, glutamate) and their corresponding metabolites of the TCA cycle (e.g. malate, oxaloacetate and α-keto glutarate) which were found to be elevated in the nephrotic rat (135,136,150) due to peripheral mobilization of amino acids in the nephrotic syndrome. They also found an increase in the activity of liver enzymes of amino acid metabolism (e.g. aspartate aminotransferase, alanine aminotransferase, tyrosine amino transferase, serine dehydratase and tryptophan oxygenate) in the nephrotic rat liver and in fed and fasted controls. The increases in transamination and deamination indicated an increase in the size of the hepatic amino acid pool or in the rate of amino acid flow. Since amino acids activate enzymes of their own catabolism, amino acids are partially diverted into the TCA cycle and their metabolites are potentially available for gluconeogenesis. They also found that nephrosis induced an increase in the incorporation of amino acid label into lipoprotein, albumin and globulin, but the label in lipids and free fatty acids was decreased suggesting that amino acids are poor precursors for lipid synthesis. The lipid was primarily supplied to the lipoprotein at the expense of cellular liver lipid, rather than from direct lipogenesis. McGole et al. (151) found that though total amino acid concentrations in renal disease did not differ significantly from control, there was a depletion of essential amino acids in uremic plasma which increased slightly after hemodialysis. The significantly higher plasma phenylalanine tyrosine ratio in renal disease was due to a deficiency in the phenylalanine hydroxylase enzyme system (152), the malabsorption of dietary tyrosine, or the bacterial decarboxylation of tyrosine to tyramine (tyramine concentration increases in uremic plasma) (153). Shear (152) studied alterations in tissue amino acid distribution in bilaterally nephrectomized rats and found an increased uptake of essential amino acids by the liver, coupled with an increased rate of RNA and protein synthesis and a decrease in the plasma concentrations of essential amino acids. Dubovsky et al. (154) reported the elevated plasma level of free proline and hydroxyproline, an index of collogen turnover in primary hyperparathyroidism and chronic uremia. Morgan et al. (156) reported elevated plasma aromatic amines in uremia, their values being roughly parallel with the elevation of BUN. The plasma amino acid concentrations of both bound and free forms in chronic renal failure have been shown to be quantitative not qualitative when compared with normal subjects (157,158,159).

Ludewig et al. (160) reported that the dietary protein intake influences the urinary protein excretion. Bloomfield (161) also had demonstrated earlier that urinary loss of protein in chronic uremia did not explain the corresponding hypoproteinemia. In numerous glomerular disorders a high degree of protein excretion (3 to 4 g/24 hour) can result in the nephrotic syndrome (162). In general, the percentage of urine protein, as albumin, can be used to predict steroid responsiveness (163).

As the concentration of serum albumin falls, the concentrations of α-2-globulins and blood lipids rise, and salt and water retention occurs. Persistent proteinuria is associated with a high incidence of renal failure and intermittent proteinuria with serious renal disease (164, 165).

Aspartic acid, citrulline, 1- and 3-methylhistidines, taurine, and bound and conjugated amino acids are known to increase in uremics, whereas other amino acid concentrations may be decreased (166). Josephson (167) suggested that improvement can be made by the addition of histidine as an essential amino acid, to achieve a positive nitrogen balance; addition of arginine was thought to be injurious.

Giordano et al. (139,141,143,145) reported that uremics, on dialysis, have in their plasma aminograms increased 1- and 3-methylhistidine, cystine, citrulline, glycine, alanine. Aspartic acid, asparagine, glutamine, serine, ornithine and notably proline and hydroxyproline increased with length of dialysis. The increase in proline and hydroxyproline was most significant in patients with bone disease.

Others have estimated uremic muscle tissue amino acids, and have found increased non-essential amino acids especially taurine, glutamic and aspartic acid and decreased branched amino acids (185,145).

G. Inhibitory Effects of Metabolites Retained in Uremia

Attempts to elucidate the influence of uremic toxins on enzyme activities at subcellular levels have been made by many workers (169). Giordano *et al*. (170) observed the inhibitory effect of urea on monoamine oxidase at the plasma concentration in chronic renal failure, but at higher concentrations (BUN 140 mg/100 ml) less enzyme inhibition occurred. Monoamine oxidase destroys serotonin and catecholamines and thus regulates amine metabolism in nervous tissue; inhibition of monoamine oxidase in uremia leads to the pathogenesis of some of the neurological disturbances observed. It is also known that urea (500 mg) inhibits oxygen uptake by brain slices. Substances other than urea acting as enzyme inhibitors at the subcellular level of the central nervous system have also been investigated (2,171,172,173). They reported the effect of phenolic acids on cerebral metabolism, on the rate of respiration, on anaerobic glycolysis of guinea pig brain slices, and on the inhibition of the activity of some selected enzymes; namely, the decarboxylases of 3,4-dihydroxyphenylalanine, 5-hydroxytryptophan, and glutamic acid, glutamic oxaloacetic transaminase, 5-nucleotidase, amine oxidase and lactic dehydrogenase. Elevated blood levels of pyruvate, acetoin and 2,3-butylene glycol indicated that ATP synthesis in uremia was diminished (174) and that the ATP deficiency induced by several retention products (e.g. tyramine) led to diminished consciousness in uremia.

The following table has the lists of some of the enzyme systems known to be affected by uremia:

SUMMARY OF ENZYME SYSTEMS AFFECTED BY UREMIA

Experimental Condition	Enzyme System	Enzyme Activity	Reference
1. Bilateral nephrectomy (rat liver enzymes 24 hours post operative)	PFK, HK, GK	Decreased	(155)
	LDH, G-6-PDH	No change	
	PK, F-1,6-DP-ase, GPT, GOT	Increased	
2. Urea or creatinine incubation (RBC-enzyme)	PK, PFK, HK	No change	(175)
3. Aminonucleoside (of puromycin) induced nephrotic rat (liver enzymes)	Glycolysis Enzymes		(176)
	GK, PK, G-6-PDH	Decreased	
	PFK, HK, Aldolase	No change	
	Gluconeogenesis Enzymes		
	G-6-P-ase, F-1,6-DP-ase	Decreased	
	PEPCK, PC	Increased	
	Amino Acid Metabolic Enzymes		
	AAT, ALAT, TAT, SDH, TPO	Increased	
	Lipogenesis Enzymes		
	NADP-malate DH, ATP-citrate lyase Acetyl-CoA carboxylase, FA synthetase	Decreased	
4. Uremic plasma toxin low M.W. (<500)	ETKA (Thiamine pyrophosphate dependent pentose phosphosphate shunt enzyme)	Depressed	(177)

(Continued)

SUMMARY OF ENZYME SYSTEMS AFFECTED BY UREMIA (Continued)

Experimental Condition	Enzyme System	Enzyme Activity	Reference
5. Urea, guanidine (0.05-0.2 M)	Xanthine oxidase; monoamine oxidase	Inhibition	(178,170)
6. 100-200 mg% BUN (uremic patients	Glucose oxidation by rat brain mice	Acceleration	(179)
7. BUN, <100 mg% and >150 mg%	LDH	Inhibition	(180)
8. 20 to 24 specific phenolic acids obtained from uremic serum or dialysates	Respiration, DOPA decarboxylase. Anaerobic glycolysis, GOT, SPT. Glutamic acid decarboxylase LDH	Inhibition	(2)
9. N-methyl-2-pyridone-5-formamido acetic acid	^{3}H-leucine incorporation into protein by cell free liver homogenate	Inhibition	(181)
10. Liver from uremic rat (BUN 181-269 mg%)	L-(u-^{14}C)-leucine uptake into protein by cell free liver extract	Acceleration	(128)
11. Uremic serum (human) (BUN 100-300 mg%, creatinine 5.4-3.0 mg%	Oxidative phosphorylation by rat liver mitochondria	Inhibition with glutamate and malate as substrate	(182)

(Continued)

SUMMARY OF ENZYME SYSTEMS AFFECTED BY UREMIA (Continued)

	Experimental Condition	Enzyme System	Enzyme Activity	Reference
12.	Uremic plasma (human) (BUN 38-222 mg%) GSA	ADP-induced platelet factor 3 activity (Stypven time, as above)	Elevation	(183)
13.	Uremic sera (kidney cortex enzyme) (RBC enzyme)	Coenzyme A and gluconeogenesis pyruvate carboxylase HK, PK, G-6-PDH, GOT	Decreased Increased	
14.	Uremic blood (an inhibitor)(a peptide)	MW <5,000 PFK, MW >5,000-10,000 ATPase	Decreased Decreased	(185)
15.	Pyelonephritis (inflammation in extra or intra renal obstruction in tubular damage)	Alkaline phosphatase (released)	Increased in serum	
16.	Sub-total nephrectomy (rat liver)	Endoplasmic reticulum oxidase and demethylase	Decreased	(185,186)

H. Lipid Metabolism

It is over one hundred and fifty years since the lactescence of chronic uremic serum was recognized (187, 188).

In non-nephrotic patients with chronic renal failure, serum cholesterol and non-esterified fatty acid concentrations are usually within the normal range, while triglycerides and total lipids are usually increased (189-193). Reimold _et al_. (193) reported that serum total cholesterol concentrations were increased due to an increase in the free fraction while the concentration of the esterified fraction was normal.

ARF following nephrectomy and other experimental procedures is associated with increased plasma total lipids, cholesterol, phospholipids, and neutral fat (194-196) and an increase in lipoprotein of the S_{10} to S_{20} and S_{20} to S_{40} range concurrent with a fall in those of S_1 to S_{10} (197). Purified kidney extracts can prevent some of these changes, suggesting that kidneys produce a substance which influences lipid metabolism (198,199).

In humans, α-lipoproteins of high density which migrates electrophoretically with α-globulin have been shown to become depressed with nephrectomy and to return to normal following successful kidney transplantation (200).

Roodvoets _et al_. (189) found that hyperlipemia in chronic renal failure was characterized by an increase in the pre-ß-lipoproteins. A major portion of the total weight of the pre-ß-lipoprotein complex is constituted by lipids, triglyceride constituting the major lipid fraction (201); the triglyceride is of endogenous origin and is synthesized mainly in the liver. Fredrickson et al. (201) found that uremic plasma constitutes a Type IV hyperlipoproteinemia and this type of lipoprotein, a "hall mark of endogenous hyperlipemia" was "typically carbohydrate inducible and accompanied by glucose intolerance". The increase in plasma triglyceride concentration, and lipoproteinemia, may be the result of increased hepatic triglyceride synthesis, diminished removal of triglyceride from plasma by adipose tissue or a combination of both. Bagdade et al. (191,192) proposed that the increased plasma triglyceride concentration in uremia is the combined result of increased synthesis and impaired triglyceride removal. The increased hepatic triglyceride synthesis is the consequence of the increased plasma insulin concentration found in the patients. They also reported that in uremia the peak post-heparin lipolytic activity, an indirect estimate of tissue lipoprotein lipase and hence triglyceride removal capacity, decreased. The insulin antagonism in uremia also causes a diminution in lipoprotein lipase activity leading to hypertriglyceridemia. In carbohydrate induced hyperlipemia carbohydrate is converted into fat and released as pre-ß-lipoproteins in plasma.

It is known that in rats glucose tolerance is impaired by high dietary sucrose and the effect is enhanced if dietary protein intake is low (202,203). Bagdade et al. (192) also supported this by reporting increased triglyceride concentrations in uremics who were maintained by dialysis and who were not on a high carbohydrate intake.

In nephrotic rats, liver metabolite patterns (e.g. accumulation of α-glycerophosphate) suggest enhanced fatty acid synthesis (204). It has been recognized that increased glycolysis is associated with lipogenesis or increased gluconeogenesis, and that amino acid catabolism is associated with enhanced fat utilization.

It has been suggested (176) that hyperlipogenesis may occur in the nephrotic rats but that it is not a prerequisite of nephrotic hyperlipedemia.

Synthesis of the apolipoprotein seems to be the determining factor. The lipogenic pathway may respond to the apolipoprotein production upon the availability of adequate dietary precursors. The lipogenic response depends on the manner of induction of nephrosis (whether by amine nucleoside or by antikidney serum) and on the severity of the disease. If lipogenesis is not increased once the apolipoprotein is synthesized the apolipoprotein may draw its lipid complement from preformed liver lipids and produce a shift in plasma/liver lipid distribution without greatly changing the size of their lipid pool.

Glucose was shown to be a good precursor of plasma lipids in nephrosis (135), and resulted in increases in some serum triglyceride but was without effect in others (205). Using in vivo and in vitro systems, many workers demonstrated that an increased supply of lipids to the circulation occurs in hepatic hyperlipodemia (35,206,207), and increased hepatic lipoprotein synthesis appears to be the primary event (208,209). Kaye *et al*. (168) reported increased accumulation of triglyceride, cholesterol, total low density lipoproteins, glycerol, glucose, and immunoreactive growth hormone, together with an increase in pre-β-lipoproteins on electrophoresis, in patients with CRF. These were considered to represent the end results of the combination of a number of factors including the most important one, the accumulation of insulin antagonists.

I. Carbohydrate Metabolism in Normal Mammals

Carbohydrate metabolism in effect commences with G-6-P rather than with glucose itself as it is from this ester, G-6-P, that the important pathways of carbohydrate metabolism originate. The five major chemical processes involving metabolic pathways are:

i. the reversible processes (glucogenesis and glycogenolysis), glucose $\rightleftharpoons$ glycogen

ii. the conversion of sugars (e.g. fructose, mannose, and galactose) into glucose,

iii. the reversible processes converting glucose to pyruvate (glycolysis and gluconeogenesis),

iv. the oxidation of pyruvate to CO_2 and H_2O in Krebs citric acid cycle,

v. the reduction of carbohydrate carbon (-CHOH-groups) to fatty acid carbon ($-CH_2-$) and the oxidation of amino acid carbon ($-CH_2-$) to carbohydrate carbon (-CHOH-), and the reverse.

Figure 15 shows the enzyme sequences of glycogenolysis, glycogenesis, the special oxidative pathway (Pentose phosphate shunt) and glycolysis, the tricarboxylic cycle and its reverse direction (in gluconeogenesis) and the points at which they integrate with the reactions concerned in the metabolism of lipid and protein.

1. Glucose Utilization

Glucose is released to the environment by hydrolysis of G-6-P by a special microsomal enzyme, G-6-Pase. G-6-P may be hydrolyzed in cells of the liver, intestine, and kidney only. The sum of this hexokinase reaction plus the G-6-Pase reaction is equivalent to hydrolysis of ATP to ADP and P_i. G-6-Pase is a complex enzyme, its activity depending on lipids of its normal milieu. It also serves as an inorganic pyrophosphatase:

$$PP_i \xrightarrow[Mg^{++}]{H_2O} 2\ P_i.$$

Kinase catalyzes the reaction:

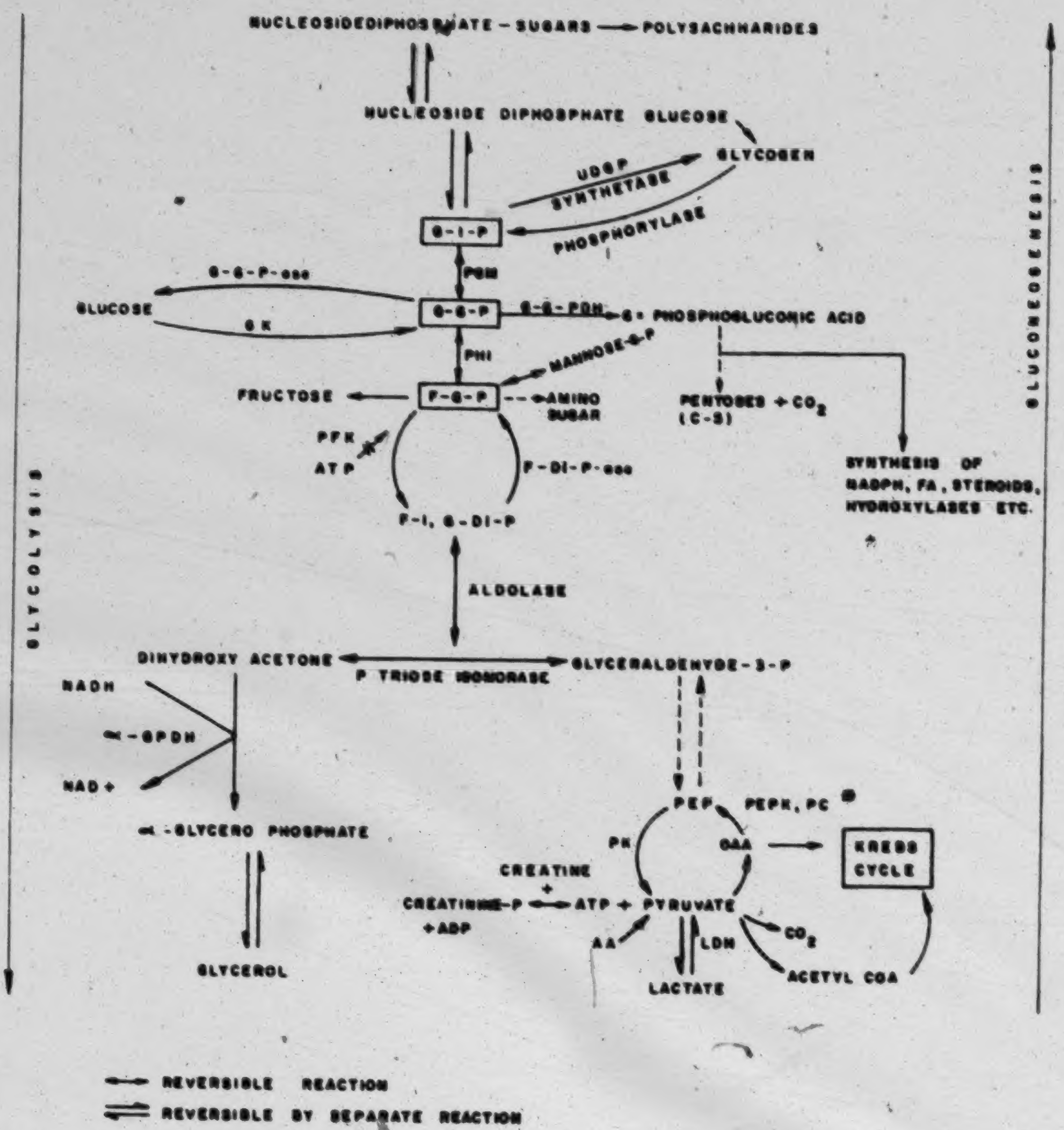

NUCLEOSIDEDIPHOSPHATE - SUGARS
POLYSACCHARIDES
NUCLEOSIDE DIPHOSPHATE GLUCOSE
GLYCOGEN
UDGP SYNTHETASE
PHOSPHORYLASE
G-1-P
PGM
G-6-P-ase
GLUCOSE
GK
G-6-P
G-6-PDH
6- PHOSPHOGLUCONIC ACID
PHI
MANNOSE-6-P
FRUCTOSE
F-6-P
AMINO SUGAR
PENTOSES + CO2 (C-5)
SYNTHESIS OF NADPH, FA, STEROIDS, HYDROXYLASES ETC.
PFK
ATP
F-DI-P-ase
F-1, 6-DI-P
ALDOLASE
DIHYDROXY ACETONE
P TRIOSE ISOMERASE
GLYCERALDEHYDE-3-P
NADH
α-GPDH
NAD+
α-GLYCERO PHOSPHATE
GLYCEROL
PEP
PEPK, PC
PK
OAA
KREBS CYCLE
CREATINE +
CREATININE-P + ADP
ATP + PYRUVATE
AA
LDH
CO2
LACTATE
ACETYL COA
GLYCOLYSIS
GLUCONEOGENESIS
REVERSIBLE REACTION
REVERSIBLE BY SEPARATE REACTION

Fig. 15. Enzyme sequences in glycolysis, gluconeogenesis, glycogenesis, and the pentose monophosphate shunt (213,214).

$$PP_i + \text{glucose} \longrightarrow \text{G-6-P} + P_i$$

This process of G-6-P formation has the potential that hepatic glucokinase has, but the supply of pyrophosphate makes this route of little physiological importance.

Conversion of G-6-P to G-1-P is the initial step in the synthesis of nucleoside diphosphate esters (adenosine, guanosine, uridine, and thymidine) of glucose. These nucleoside diphosphate esters of sugars are utilized for the synthesis of a wide variety of polysaccharides (e.g. glycogen, amylose, etc.).

The hexose monophosphate shunt (phosphogluconate oxidative pathway or the pentose phosphate shunt) is operated by cytoplasmic enzymes (cytoplasma of those cells in which they function) (e.g. G-6-PDH, 6-phosphogluconic acid DH, epimerases, transketolase, transaldolase) through a series of reactions. The initial step in the formation of pentose is the oxidation of G-6-P at C-1 (while glycolysis and TCA can use glucose at C-1 and C-6) to form 6-phosphogluconic acid. This is a special oxidative pathway (second only to the glycolytic pathway, the other major pathway) by which total combustion of glucose can occur independently of the TCA cycle; NADPH is generated by this pathway for use in the synthesis of fatty acids, various hydroxylases, and steroids, and serves as a source of D-ribose, and 4-carbon and 7-carbon sugars. In liver more than 30% of the CO_2

arising from glucose stems from this pathway. Liver, mammary gland, testis and the adrenal cortex are active sites for this pathway. Striated muscle does not have this pentose phosphate shunt; the glycolytic and TCA pathways are only pathways in muscle for glucose metabolism.

Conversion of G-6-P to F-6-P which is then phosphorylated to F-1,6-diphosphate, is probably the most noteworthy fate of G-6-P. This is the initial event leading to glycolysis (Embden-Meyerhof pathway) through which the energy of the glucose molecule is made available to the cells as ATP. It leads to complete oxidation of glucose via Krebs cycle.

Glycolysis also yields some energy in the form of ATP for utilization during muscle contraction. This glycolysis provides a means for a rapidly obtaining ATP in muscle, a relatively anaerobic organ. This is particularly important during sudden demand for energy to be made available in excess of that produced by oxidative processes, as in the case of strenuous exercise.

2. Energy Production from the Metabolism of Carbohydrate

In glycolysis, only 2 molecules of ATP are synthetized. Most ATP is produced during the oxidation of hexose via the TCA cycle. Two moles of ATP are used in the metabolism of a mole of glucose and only one ATP is utilized per hexose unit if glycogen is metabolized. Therefore, there is a net production of 39 moles of ATP per glycogen hexose unit metabolized. If glycolysis is purely anaerobic, the ATP production is reduced by 6 moles per mole of hexose metabolized. Therefore, the degree of anaerobiosis and the amount of lactic acid production will determine net energy production of glycolysis.

3. Glucogenesis and Glycogenesis

These are the processes of conversion of substances to glucose and glycogen by the reversal of glycolytic process. Pyruvic acid (produced from many substances) occupies a key position in the formation of glucose and glycogen by reversal of glycolytic reactions. This was first suggested by A.V. Hill, who observed that an isolated muscle contracting anaerobically could convert glycogen to lactic acid, but that in the presence of oxygen, lactic acid disappeared with 20% being oxidized to CO_2 and the remainder being converted to glycogen. Under this condition, or in the liver receiving blood lactic acid from skeletal muscle, the adequate supply of CO_2 leads to the synthesis of ATP. This occurs via mitochondrial oxidative phosphorylation of pyruvate and oxidation of lactate to pyruvate; where the pyruvate kinase reaction is readily reversible, the pyruvate could be converted to phosphoenolpyruvate and thence to hexose by reversal of the glycolytic reaction. However, reversal of the pyruvate kinase reaction occurs slowly and, therefore, some alternate pathway from pyruvate to PEP is required if lactate or pyruvate from other metabolic sources is to be converted to hexose. Such a pathway, and its related enzymes were discovered in the anaplerosis process of Konberg (210,211) in which the supply of OAA is replenished by CO_2 fixation with pyruvic acid (or malic acid in the presence of MDH) catalyzed by PEP carboxylase, in muscle and liver (210,212).

$$\text{PEP} + \text{CO}_2 + \text{IDP} \xrightarrow{\text{Mg}^{++}} \text{OAA} + \text{ITP}$$

Direction is given to the process by ATP and acetyl CoA which arose in the mitochondrial metabolism of pyruvate. OAA is reduced by mitochondrial MDH to malate which diffuses into the cytoplasma to be reoxidized to oxaloacetate by cytoplasmic MDH. PEP-carboxykinase (activated byacetyl-CoA) then utilizes the energy of ITP or GTP as it catalyzes the formation of PEP (see figure 15). PEP may then be converted to FDP by reversible reactions. NADPH required for reduction of 1,3-diphosphoglycereate is provided by concurrent oxidation of lactate to pyruvate.

The prevailing low concentrations of ADP and AMP and the high concentrations of ATP and citrate would inhibit PFK and activate DPFPase, facilitating formation of G-6-P which can then be hydrolysed by G-6-P-ase (213,214).

Metabolism of odd carbon fatty acids leads to the production of some liver glycogen. In the final stage of the β-oxidation of these acids one mole of propionic acid is formed. This is a glycogen former through preliminary oxidation to pyruvic acid followed by reversal of glycolysis. All members of the TCA cycle and all substances convertible into TCA cycle members form glycogen in the body. For example, conversion of succinic acid to glycogen occurs through successive conversions: succinic acid → fumaric acid → malic acid → oxaloacetic acid → pyruvic acid → glycogen. At three stages, glycolytic reactions are not readily reversible; they present blocks to glucose synthesis through pyruvate:

(a) Glucose + ATP → G-6-P + ADP

This block can be removed by hydrolysis of G-6-P by liver G-6-P-ase and this block is not involved in glycogen synthesis (215).

(b) The Reaction from F-6-P to F-1,6-DP is in Essence Reversible and the Block can be Removed by Hydrolysis of F-1,6-DP to F-6-P by F-1,6-DP-ase.

(c) The Third Block is in the Reaction:

$$\mathrm{PEP + ADP} \xrightleftharpoons{\mathrm{PK}} \text{hydroxypyruvate} \rightleftharpoons \text{pyruvate}$$

The free energy decrease of this reaction, ΔF^{-}, is about

6 KCal which means that the equilibrium lies far to the right. Therefore, phosphorylation of pyruvate to PEP for glycogen synthesis is opposed by a large energy barrier (211).

In muscle, glycogen synthesis from pyruvate does not proceed through this reversible reaction (216), but in liver, PEP is formed chiefly by a shunt mechanism (216) as follows:

$$\text{Pyruvate} + \text{NADPH} + \text{H}^+ + \text{CO}_2 \overset{\text{MDH}}{\rightleftharpoons} \text{malate} + \text{NADP}^+$$

$$\text{Malate} + \text{NAD}^+ \overset{\text{MDH}}{\rightleftharpoons} \text{oxaloacetate} + \text{NADH} + \text{H}^+$$

$$\underline{\text{Oxaloacetate} + \text{ITP} \rightarrow \text{PEP} + \text{CO}_2 + \text{IDP}}$$

$$\text{SUM: Pyruvate} + \text{NADPH} + \text{H}^+ + \text{NAD}^+ + \text{ITP} \rightarrow \text{PEP} + \text{NADH} + \text{NADP}^+ + \text{IDP}$$

This provides the means of overcoming the barrier. The 6 KCal of energy per mole required to phosphorylate pyruvate is supplied by linking the endergonic reaction with exergonic reactions (see above) which increase NADPH, NAD^+ and ITP and decrease NADH, NADP^+ and IDP to drive the reaction to the right with the formation of PEP.

4. Conditions Under Which Tissue Glycogen Accumulates

Glycogen accumulates only during resting periods in well fed animals with a liberal supply of food. Due to minimal oxidative requirements, muscle tissue forms glycogen by reversal of glycolysis and the TCA cycle. In exercise, oxidative energy requirements are highest while in starvation when the food supply is cut off blood glucose levels are low and only small amounts of glycogen are present in liver and muscle. In the liver in starvation, gluconeogenesis, by reversal of glycolysis and the TCA cycle, forms glucose from glucogenic substances and amino acids. Even in diabetes this ability of the liver remains unimpaired.

5. The Fate of Pyruvic Acid (See Figure 16)

Like G-6-P, pyruvic acid occupies a key position in metabolism. It is the end product in the main line of glycolysis and from it acetyl CoA is formed to run the TCA cycle, and to synthesize fatty acids and cholesterol. Pyruvic acid, by reversible reduction, forms lactic acid which can be converted back to glucose, used for the formation of oxaloacetate or malic acid, or trans-aminated to form alanine. Alanine formation is also a reversible process, that is, alanine by deamination forms pyruvic acid. Several acids in their metabolism are directly converted to pyruvic acid. Decarboxylation of oxaloacetic acid forms pyruvic acid as does oxidation of malic acid by MDH.

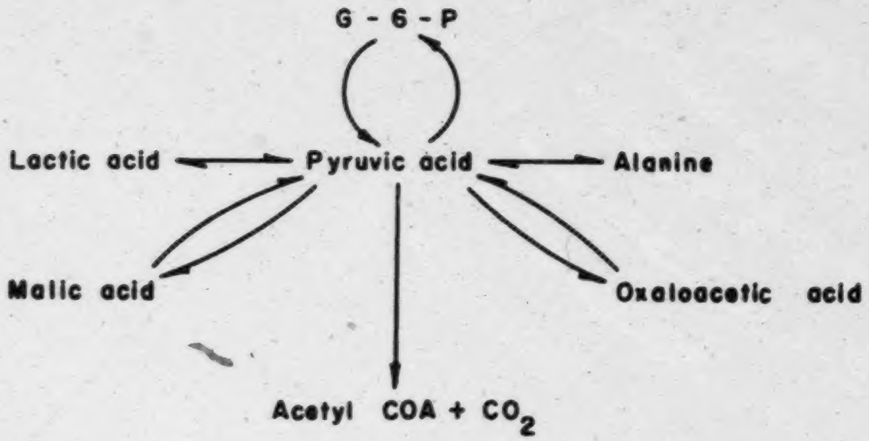

G - 6 - P
Lactic acid
Pyruvic acid
Alanine
Malic acid
Oxaloacetic acid
Acetyl COA + CO2

Fig. 16. Fate of pyruvic acid.

In muscle tissue, synthesis of glycogen occurs mainly by direct phosphorylation of pyruvate, while in the liver synthesis of glucose and glycogen occurs mainly via malate and oxaloacetate and PEP; the pyruvate carboxylase level is high. Anaplerosis is the mechanism for supply of OAA (from amino acids) to initiate succeeding turns of TCA cycle. The major fate of pyruvic acid in most mammalian cells is oxidation to CO_2 and acetyl CoA.

6. Glycogen Metabolism

Glycogen, the polysaccharide form for carbohydrate storage, occurs in small amounts in most tissues, notably liver, skeletal muscle, and adipose tissue cells.

In tissue cells glycogen is bound with proteins, and exists in two forms; desmoglycogen, an insoluble form bound to proteins by "residual valencies", and lyoglycogen, a free soluble form (217). Meyer et al. (218,219) suggested the binding of glycogen to protein is physical not chemical, that is, an entrapping of protein by the glycogen chains.

(a) The Molecular-Structure of Glycogen

i. Molecular weight. Physico-chemical measurements such as osmotic pressure, viscosity, sedimentation, diffusion and end group assay by chemical means are methods used to determine the molecular weight and structure of glycogen. That glycogen has a varied molecular weight [(2-6) x 10^6 with ~2,500-α-D-(1-6) interchain linkages] was first published in 1931 (136) and later reviewed (349).

ii. Enzyme methods for structural analysis. Glycogen is attacked by amylases, and phosphorylases, and limit dextrin by Amylo-1,6-glucosidase. Amylases are of two types, α and β. α-amylase catalyzes random hydrolysis of the α-D(1→4) linkages in both exterior and interior chains of glycogen, giving maltose (220); β-amylase catalyzes a stepwise hydrolysis of alternate linkages in a chain of α-(1→4) linked D-glucose residues liberating maltose (221). β-amylase action starts at the non-reducing end of the chain and ceases when glucosidase linkages other than α-D(1→4) are encountered, and is confined to the exterior portions of the chain producing high molecular weight limit-dextrin (β-dextrin or maltose) which contains all the inter-chain linkages.

Phosphorylases (222) in the presence of inorganic phosphate, remove D-glucose residues from the exterior chains of glycogen.

$$[G]_n \rightarrow [G]_{n-1} + G\text{-}1\text{-}P.$$

Phosphorylase can not by-pass interchain linkages and its affinity for glycogen depends on its source (232,223); for example, muscle phosphorylase has more affinity for glycogen and yields 30-50% D-glucosyl phosphate.

Amylo-1,6-glucosidase, which has no action on glycogen itself, hydrolyses the 1,6-glucosidic bond of limit dextrin (224) obtained from glycogen by β-amylase:

iii. Periodate oxidation for end-group assay. In this method, formic acid which arises only from non-reducing terminal groups of glycogen is determined by titration with sodium hydroxide. Modifications using metaperiodate at a temperature of 2-20° for 1-7 days have been made and used (233,225).

iv. Methylation for end-group assay: In these studies, acid hydrolysates of gram quantities of methylated glycogen are analysed by chromatographic methods for tetra-o-methyl-D-glucopyranose which can come only from non-reducing end groups (234).

Glycogen contains equal numbers of non-reducing end groups and 1,6-interchain linkages and the determination of either proportion will facilitate the calculation of the average chain length.

(b) Tissue Distribution of Glycogen

Normally mammalian liver contains 2 to 8% glycogen on a wet weight basis. Total body carbohydrate as glucose in the average man is normally 20 to 30 g whereas in the liver glucose as glycogen amounts to more than 300 g. The percentage of glycogen in the liver is higher than in muscle, but because of larger muscle mass, its quantity is much greater in muscle (226).

The rate of tissue glycogen formation depends on the following factors:

i. the rate of intestinal absorption.

ii. the metabolism of the substances absorbed.

iii. physiological changes in the animals. For example, rats fasted 48 hours form glycogen more rapidly from glucose than from galactose, but if kept on an ordinary diet the reverse is true. Prolonged starvation decreases the animals' capacity to oxidize glucose and under this condition animals convert much of their own glycogen. High protein diets maintain tissue glycogen much better during starvation. The protein effect stimulates gluconeogenesis which is depressed by a high carbohydrate diet. Animals fasted for long periods establish better gluconeogenesis from tissue protein and yield larger quantities of glucose to the tissues.

iv. various patho-physiological conditions either deplete or increase the glycogen content of the tissues (227,228).

v. epinephrine and glucagon administration causes depletion of liver glycogen, by activating cyclic 3',5'-AMP and by converting phosphorylase 'b' to 'a' thus making G-6-P available for increased blood glucose levels (hyperglycemia); this causes a fall of muscle glycogen, a rise of blood lactate and liver glycogen.

vi. insulin insufficiency leads to reduced activity of the glycogen synthetase system, reduced acetyl CoA (which stimulates PEPCK for the synthesis of PEP in gluconeogenesis), reduced GK activity, accelerated G-6-P-ase activity, and decreased liver glycogen formation. Administration of insulin usually increases muscle glycogen but not liver glycogen. Adenohypophyseal hormone tends to increase liver glycogen. Epinephrine, glucagon and adenohypophyseal hormones are antagonistic to insulin action and inhibit glucose utilization.

vii. glucocorticoids (cortisol and its derivatives) produce a glycogenic effect; that is, they enhance glycogen content by stimulating gluconeogenesis from amino acids. Adrenalectomized animals when starved or placed under stress can not maintain blood glucose and tissue glycogen levels as they fail to form glucose from protein. Administration of adrenocorticoids to well fed animals stimulates enhancement of liver glycogen.

viii. acidosis causes rapid glycogenolysis with hyperglycemia and glucosuria, and reduces liver glycogen.

ix. tissue anoxia (e.g. asphyxia or ether anaesthesia) causes both liver and muscle glycogenolysis and also produces tissue acidosis. Oxygen is necessary for glycogen synthesis. Muscle glycogen, unlike liver glycogen is not depleted by fasting, but convulsions or exercise will deplete it.

(c) Hereditary Disorders of Glycogen Metabolism or Glycogen Storage Disease

Hereditary disorders are characterized by accumulation of tissue glycogen and based on the lack of all enzymes of glycogen metabolism. The lack of liver G-6-P-ase, (Von Gierke's disease) (229) which causes hypoglycemia, the lack of debranching or branching enzyme which leads to the accumulation of glycogen with abnormal branch length, the lack of muscle phosphorylase (Mcardle's disease) (230) and the lack of liver phosphorylase enzymes (Hers disease) (231) impair the body's capacity to hydrolyse glycogen. Thus, the accumulation of glycogen in tissues can be correlated with a relative deficiency of either G-6-P-ase or debranching enzyme or phosphorylase or glycogen synthetase (235-237,8). Figure 17 outlines the metabolic blocks of glycogen cycle enzymes due to disturbances of glycogen metabolism.

(d) Glycogenolysis and Glycogenesis

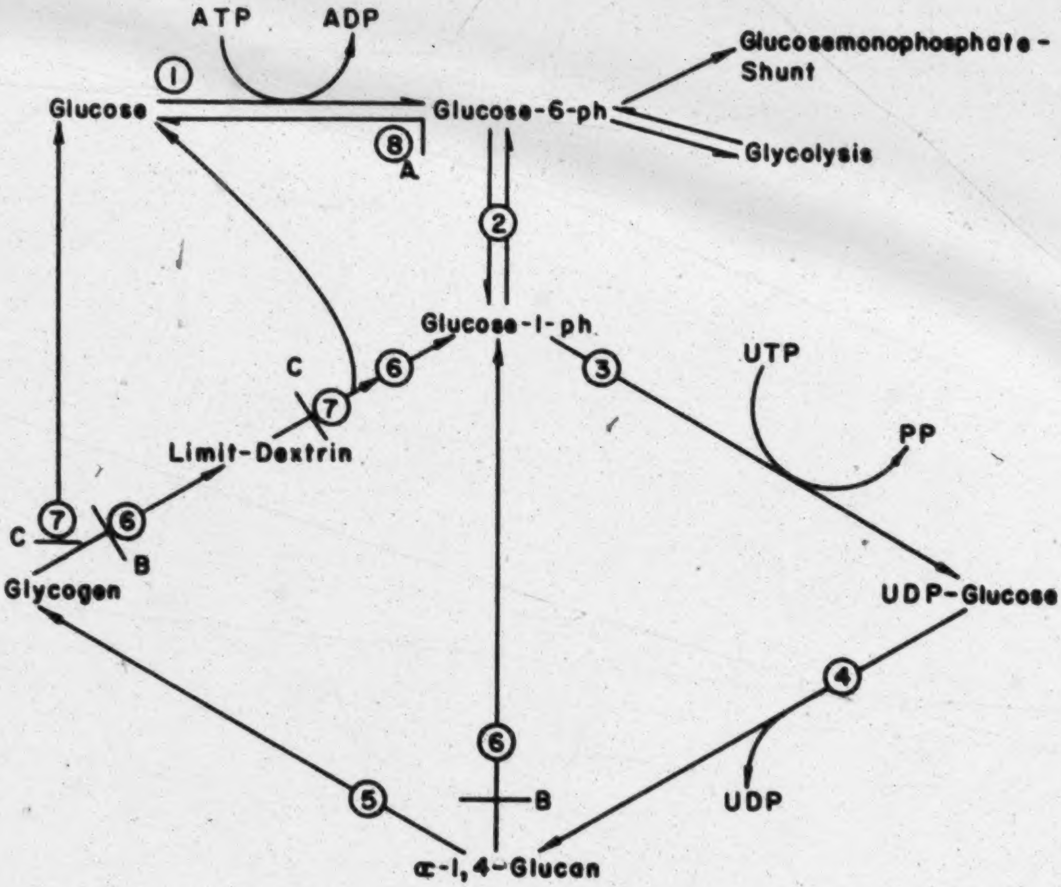
Glycogenolysis implies breakdown of glycogen, and occurs by hydrolytic processes within the cells. G-1-P thus produced is converted to G-6-P by the PGM reaction and enters main pathways of carbohydrate metabolism. G-6-P-ase, a microsomal enzyme, converts G-6-P to glucose only in the intestine, liver and kidney and thus releases glucose to the circulating blood. Cori and Larner (232) suggested the existence of a phosphorolytic pathway by

Fig. 17. Disturbances of the glycogen metabolism. Metabolic blocks in (A) v. Gierke's disease, (B) Hers' disease, and (C) Limit dextrinosis. Enzyme reactions:

1. Glucokinase (2.7.1.2)
   ATP + D-glucose → ADP + D-glucose-6-ph.
2. Phosphoglucomutase (2.7.5.1)
   D-glucose-6-ph + D-glucose-1,6-diph
   $\rightleftharpoons$ D-glucose-1-ph + D-glucose-1,6-diph.
3. Glucose-1-phosphate uridylyltransferase (2.7.7.9)
   UTP + D-glucose-1-ph → UDP-glucose + PP.
4. UDP-glucose-glycogen glucosyltransferase (2.4.1.11)
   UDP-glucose + $(\alpha\text{-}1,4\text{-glucosyl})_n$ →
   UDP + $(\alpha\text{-}1,4\text{-glucosyl})_{n+1}$.
5. α-Glucan-branching glycosyltransferase (2.4.1.18)
   Catalyzes the branching of polysaccharide chains by transferring part of a 1,4-glucan chain from a 4- to a 6-position.
6. α-Glucan phosphorylase (2.4.1.1)
   $(\alpha\text{-}1,4\text{-glucosyl})_n$ + P → $(\alpha\text{-}1,4\text{-glucosyl})_{n-1}$
   + D-glucose-1-ph.
7. Amylo-1,6-glucosidase (Dextrin-1,6-glucosidase (3.2.1.33).

   Hydrolyzes 1,6-glucosidic links at the branching points; formation of free glucose.
8. Glucose-6-phosphatase (3.1.3.9)
   D-glucose-6-pH → D-glucose + P (247).

5


ATP
ADP
①
Glucose
Glucose-6-ph
Glucosemonophosphate-Shunt
Glycolysis
⑧
A
②
Glucose-1-ph
C
⑦
⑥
③
UTP
PP
Limit-Dextrin
⑦
⑥
C
B
Glycogen
UDP-Glucose
④
UDP
⑥
B
⑤
α-1,4-Glucan

which glycogen is degraded (see figure 18) into a mixture of G-1-P (93%) and glucose (7%) by the combined action of phosphorylase and amylo-1,6-glucosidase. G-1-P is released by phosphorolysis of 1,4-glucosidic linkages and glucose is released by hydrolysis of the 1,6-glucosidic bonds. In the absence of amylo-1,6-glucosidase, the phosphorolysis of the main outer chain stops at some distance from the branching point, where the side chains are reduced to a single glucose unit. G-6-P obtained from G-1-P is then either oxidized through the pentose phosphate cycle, or converted to pyruvate by glycolysis.

(e) The Regulation of Glycogenolysis and Glycogenesis

Both the liver and muscle contain phosphorylase. Phosphorylase causes addition of glucose residues to the non-reducing ends of the branches of the activating polysaccharide to lengthen them, and it also splits glucose units from the non-reducing ends. Muscle phosphorylase acting on G-1-P does not form branched glycogen, but rather a straight chain amylose containing 80 to 200 glucose units. Phosphorylase action is limited to the formation and splitting of α-1,4-glucosidic bonds. In order to synthesize glycogen in vitro by the phosphorylase reaction it is necessary to prime the reaction by adding glycogen or another branched chain polysaccharide such as amylopectin, or a branched chain dextrin. The combined

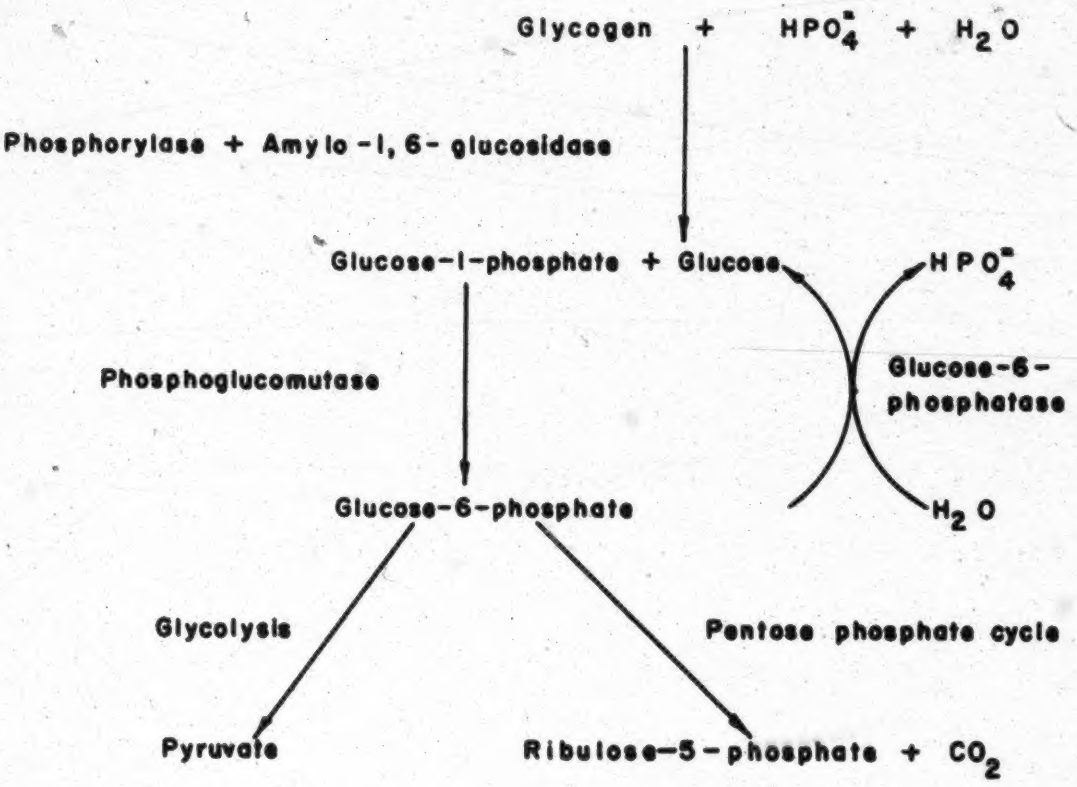

Glycogen + HPO$_4^=$ + H$_2$O
Phosphorylase + Amylo-1,6-glucosidase
Glucose-1-phosphate + Glucose
HPO$_4^=$
Glucose-6-phosphatase
H$_2$O
Phosphoglucomutase
Glucose-6-phosphate
Glycolysis
Pentose phosphate cycle
Pyruvate
Ribulose-5-phosphate + CO$_2$

Fig. 18. The phosphorolytic pathway of glycogen degradation.

action of phosphorylase and the branching enzyme upon G-1-P results in the formation of glycogen. Phosphorylase produces chains with 1,4-glucose linkage and then the branching enzyme acts upon these chains to convert some of the 1,4-linkages to 1,6-linkages thus forming branches (238). In the fed resting animal depositing glycogen, the tissue contains low concentrations of G-1-P and high concentrations of P_i favoring glycogenolysis.

Muscle glycogen phosphorylase is of two types 'a' and 'b'; phosphorylase 'a', a tetramer with a molecular weight of 500,000 and 'b', a dimer with a molecular weight of 250,000. Phosphorylase 'a' has 4 identical polypeptide chains each containing a serine residue esterified through a hydroxyl group to phosphate, and a lysine residue with its amino group as the Schiff-base (-N≡CH) of pyridoxal phosphate. The removal of tightly bound pyridoxal phosphate with cysteine makes it inactive. The phosphorylated serine and the peptide sequence of the phosphorylase 'a' is identical to that of glycogen synthetase, pointing out the possibility of their common ancestral origin.

The dimeric phosphorylase 'b', the inactive form, is obtained by removal of the phosphate group in the serine residue by phosphorylase phosphatase. AMP serves as a positive allosteric modifier of phosphorylase 'b'. Phosphorylase 'b' can be activated to phosphorylase 'a' by:

i. phosphorylase kinase which exists in active phosphorylated and inactive dephosphorylated forms. The inactive form is activated by ATP and Mg^{++}. Phosphorylase kinase is stimulated by glycogen.

ii. phosphorylase kinase which is activated by cyclic AMP (10^{-8}M). Phosphorylase 'b' kinase is like glycogen synthetase activated by a "protein factor" which needs elevated [Ca^{++}] for activity. Resting muscle, because of high ATP and low AMP concentrations, can readily convert glucose to glycogen; contraction, by utilizing ATP and generating AMP, reverses this relationship (i.e. glycogen phosphorolysis becomes maximum). Available G-6-P enters the glycolytic pathway leading to ATP synthesis and there is a demand for production of additional G-6-P for glycogen.

The increase in [AMP], and a decrease in [ATP] and [G-6-P] stimulates operation of phosphorylase 'b'. Epinephrine induces formation of 3',5'-cyclic AMP from ATP; this AMP then activates phosphorylase kinase kinase which in turn activates phosphorylase kinase stimulated by still abundant glycogen; ultimately phosphorylase 'b' is thus converted to phosphorylase 'a' permitting maximum glycogen phosphorolysis. Phosphorylase phosphatase also converts phosphorylase 'a' to 'b' and as the concentration of ATP is restored and that of AMP declines, glycogen phosphorolysis declines markedly, permitting restoration of the glycogen stores.

Liver glycogen phosphorylase works and is controlled similarly to that of muscle phosphorylase except that they have separate, independent gene control. Reactivation of liver phosphorylase is stimulated by epinephrine and also by glucagon, and ACTH, both of which have no effect on the muscle phosphorylase system (see figure 19) (7,239-241).

(f) Glycogen Synthetase

Convincing evidences suggested that glycogen synthesis occurs by the concurrent action of two transglucosylases, glycogen UDP-glucosyl transferase (also called 'transferase' or 'synthetase') (247,242), and a branching enzyme. UDP-glucose glycogen glucosyltransferase irreversibly transfers an α-D-glucosyl residue from UDP-G to an acceptor (primer-glycogen or amylopectin) containing 1→4 linked α-D-glucose residues.

Leloir et al. (247) discovered glycogen synthetase in rat liver and muscle. That the enzyme is bound to glycogen, and not liver cell structures, was confirmed by EM (243). Villar-Palasi et al. (244) proposed the presence of a glycogen cycle as glycogen metabolizing tissue contains PMG, UDPG-pyrophosphorylase (245), glycogen-UDP-glucosyl transferase and phosphorylase (see figure 20). In rat tissues, the levels of the individual enzymic activities are compatible with such a cycle as they are sufficient for in vivo glycogen synthesis. There is, for example, general agreement between the glycogen content of

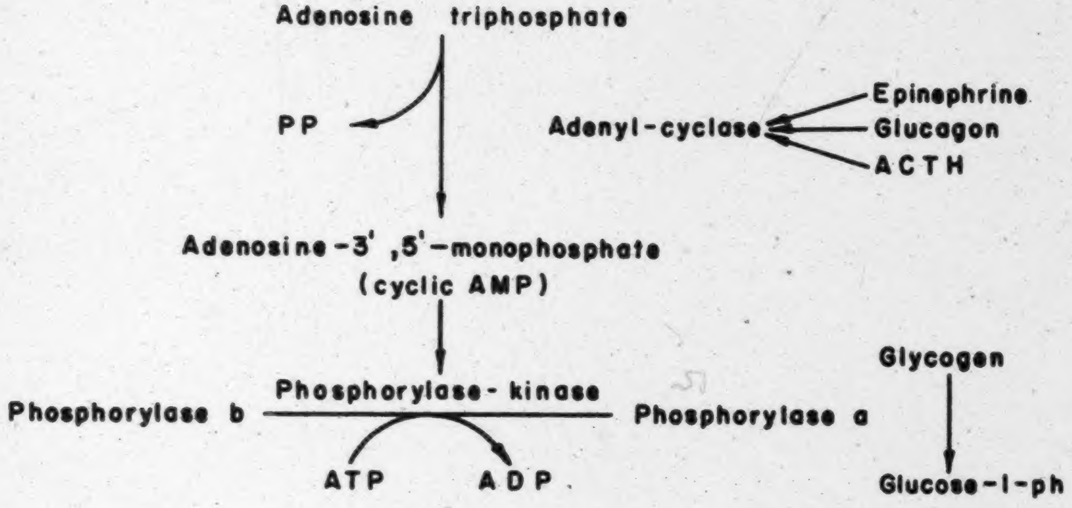

Adenosine triphosphate
PP
Adenyl-cyclase
Epinephrine
Glucagon
ACTH
Adenosine-3',5'-monophosphate
(cyclic AMP)
Glycogen
Phosphorylase-kinase
Phosphorylase b
Phosphorylase a
ATP
ADP
Glucose-l-ph

Fig. 19. Activation of phosphorylase (α-glucan phosphorylase) by epinephrine, glucagon and ACTH.

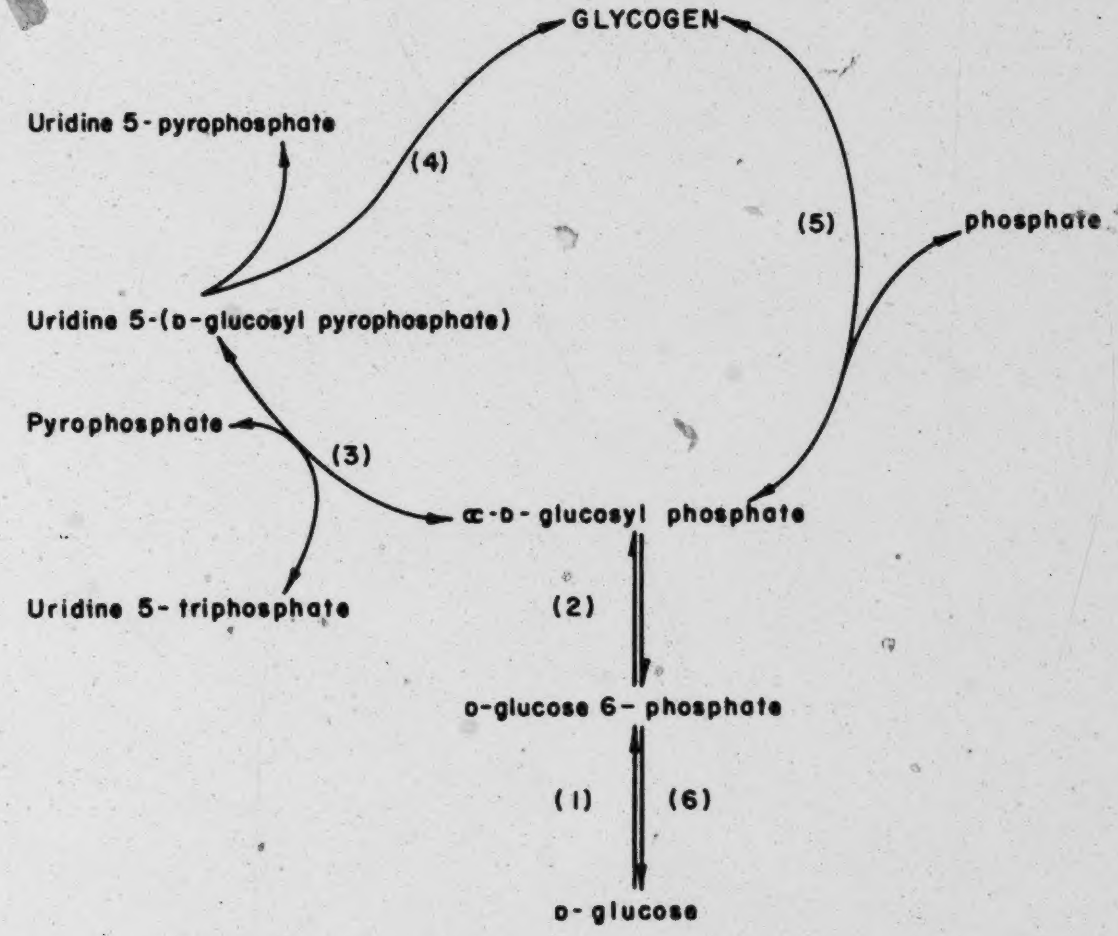

GLYCOGEN
Uridine 5-pyrophosphate
(4)
(5)
phosphate
Uridine 5-(D-glucosyl pyrophosphate)
Pyrophosphate
(3)
α-D-glucosyl phosphate
Uridine 5-triphosphate
(2)
D-glucose 6-phosphate
(1)
(6)
D-glucose

Fig. 20. Interconversion of glycogen and D-glucose by way of glycogen cycle. (Key: (1), hexokinase-adenosine 5-triphosphate; (2), phosphoglucomutase; (3), UDPG-pyrophosphorylase; (4), glycogen-UDP-glucosyl transferase and branching enzyme; (5), phosphorylase and amylo-1,6-glucosidase; (6), D-glucose 6-phosphatase).

the tissues and the above enzymic activities (245). However, the distribution of the glycogen cycle requires investigation, since histochemical studies (245) of rat skeletal muscle have shown that UDP-glucosyl transferase activity is concentrated in the small red fibres, whereas phosphorylase and the branching enzyme are most active in the large white fibres.

Glycogen synthetase catalyzes formation only of α-1,4 bonds. The interchain linkages (branching) in glycogen are formed by the action of a second transglucosylase called 'glycogen branching enzyme', amylo-(1,4→1,6)-trans-glucosylase which is present in liver, muscle, and brain. This transglucosylase removes terminal fragments of 6 or 7 glucose units from the main chain, or from the ends of major branches of the glycogen chain, at α-1,4 linkages and transfers them to the same of another glycogen molecule, but in an α-1,6-linkage; thus new branches are being created. It is the 'specificity' of this 'branching enzyme' that determines the interbranch distance along the glycogen chain (246,238).

Glycogen synthetase exists in two forms: the 'D' or phospho form which is active in presence of G-6-P and the 'I' or dephospho form which is independent of G-6-P. G-6-P stabilizes glycogen synthetase against alkaline denaturation and heat.

Enzymatically active dephospho enzyme can be phosphorylated to form the phospho enzyme by the specific glycogen synthetase kinase in presence of ATP; it is less active, but can be markedly stimulated by G-6-P. The phospho enzyme show species specificity (for example, dog phosphoenzyme needs G-6-P for activity).

Free UDP which could accumulate if the supply of both ATP and glucose is limited inhibits the phosphoenzyme strongly.

Glycogen synthetase kinase can exist in active and inactive forms. The inactive form is activated by cyclic AMP which in turn is formed from ATP by an enzyme system activated by epinephrine. In sudden stress epinephrine transforms 'I' to 'D' to facilitate glycogen formation only if the G-6-P concentration is sufficiently high to activate the synthetase as well as to form G-1-P. In muscle the kinase may also be activated by a 'protein factor' by an elevated intramuscular [Ca^{++}], which is increased during contraction.

Glycogen synthetase phosphatase, which converts 'D' (phosphoenzyme) to 'I' (dephosphoenzyme), is inhibited by glycogen itself. Consequently, if heart or skeletal muscle glycogen is depleted the 'I' form dominates; as the glycogen concentration increases, the 'D' form increases.

Again, the diminished glycogen concentration releases transferase phosphatase and consequently the 'D' form is converted to the 'I' form leading to accelerated synthesis of glycogen. Phosphorylase phosphatase also converts phosphorylase 'a' to 'b'. As the concentration of ATP is restored and that of AMP declines, glycogen phosphorolysis declines markedly, permitting restoration of glycogen stores.

4. The Sources and Fate of Blood Glucose (see Figure 21)

Normal blood glucose concentrations 8 to 12 hours after meals are 70 to 90 mg%. Blood glucose originates from diets, and from the hydrolysis of G-6-P (glycolytic product) in liver, kidney, and intestine. G-6-P may be obtained by either glycolysis, glucogenesis (from carbohydrate other than glycogen) and gluconeogenesis from non-carbohydrate precursors (e.g. amino acids, intermediate of glycolysis, glycerol, etc. i.e. glucogenic substances). The latter is the reversal of glycolysis in which enhanced activity of PC, PEPCK and fructose-diphosphatase is seen.

Glucagon from the pancreas, and epinephrine from the adrenal medulla cause glycogenolysis. Glucocorticoids increase the supply of amino acids to be metabolized to pyruvate, thus providing raw materials for gluconeogenesis (see figure 22). Insulin does the opposite; it facilitates entry of glucose into cells and exerts a suppressive influence on the synthesis of pyruvate carboxylase, PEPCK, and F-DP-ase. Insulin also increases amounts of GK in

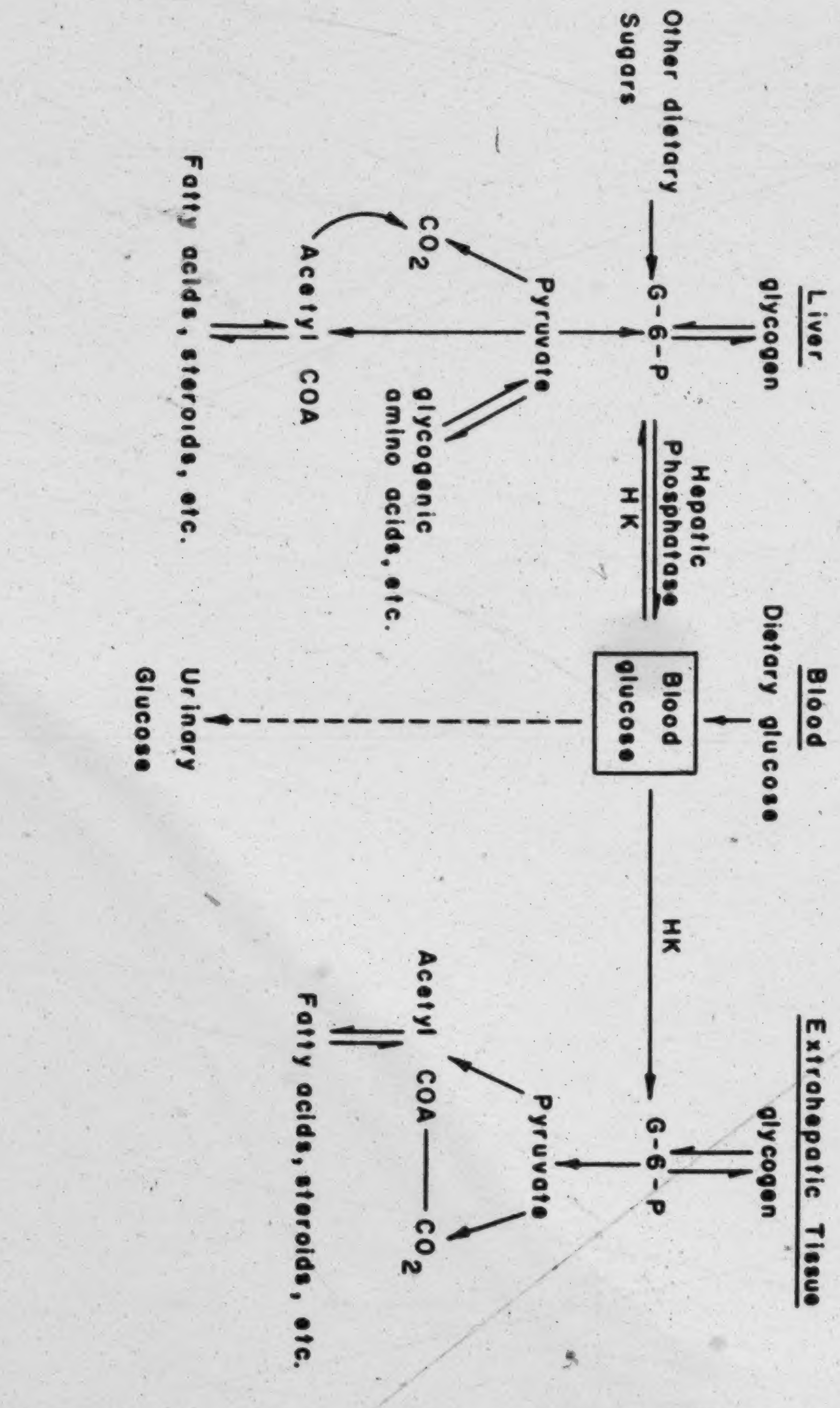

Liver
glycogen
G-6-P
Other dietary
Sugars
Pyruvate
CO_2
Acetyl COA
glycogenic
amino acids, etc.
Fatty acids, steroids, etc.
Hepatic
Phosphatase
HK
Blood
Dietary glucose
Blood
glucose
Urinary
Glucose
HK
Extrahepatic Tissue
glycogen
G-6-P
Pyruvate
Acetyl COA
CO_2
Fatty acids, steroids, etc.

Fig. 21. Sources and fate of blood glucose (213,214).

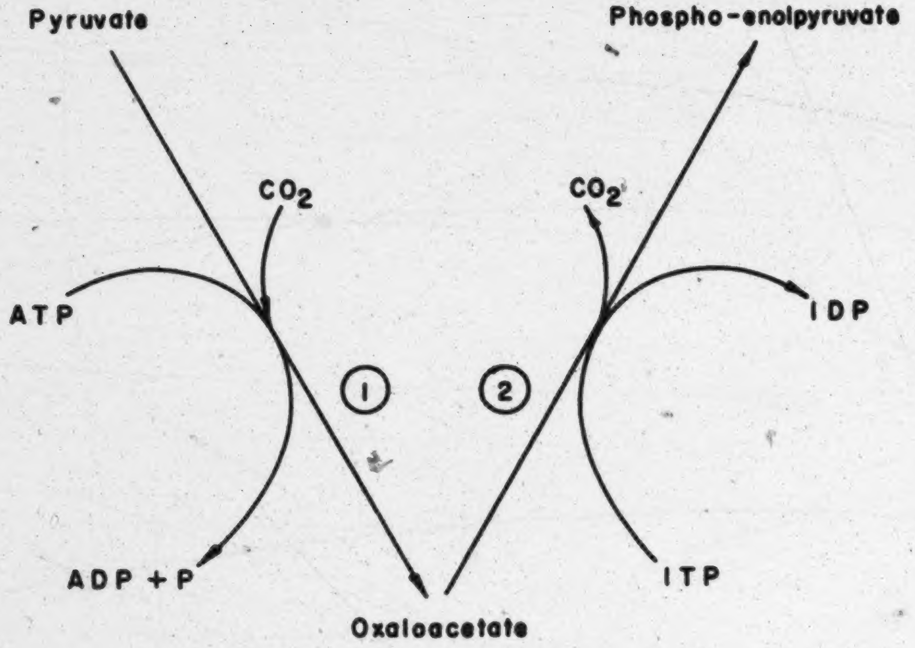

Pyruvate
Phospho-enolpyruvate
CO_2
CO_2
ATP
IDP
①
②
ADP + P
ITP
Oxaloacetate

Fig. 22. Formation of phospho-enolpyruvate from pyruvate. Enzyme reactions:

1. Pyruvate carboxylase (6.4.1.1)
   ATP + pyruvate + CO_2 + H_2O → ADP + P + oxaloacetate
   (requires acetyl-CoA as an activator).
2. Phosphopyruvate carboxylase (4.1.1.32)
   ITP + oxaloacetate → phospho-enolpyruvate + CO_2 + IDP (7).

liver and adipose cells, enhances glycogen synthetase activity and increases operation of the phospholuconate pathway to provide NADPH for increased fatty acid synthesis from either glucose or pyruvate. Insulin also promotes protein synthesis from amino acids and thus reduces gluconeogenic precursors.

J. Integration of Carbohydrate, Fat and Protein Metabolism

Final oxidation of fatty acids through oxidation of acetyl CoA, and carbohydrate through oxidation of pyruvic acid occurs through the TCA cycle. The amino acids, leucine, tyrosine and phenylalanine, are deaminated and oxidized to acetoacetic acid which then is oxidized through TCA cycle; acetyl CoA formed from these amino acids is used for the synthesis of fatty acid and cholesterol.

Alanine, cystine, and serine form pyruvic acid, which is then either oxidized through this TCA cycle or converted to glucose and glycogen by the reversal of glycolysis, or is converted to acetyl CoA for utilization as fatty acid and cholesterol.

Aspartic and glutamic acids directly enter the TCA cycle at OAA or α-keto glutarate and are oxidized completely by 2 or more turns of the TCA cycle; this leads to pyruvate, with subsequent production of glucose and glycogen. Through pyruvate, it also leads to acetyl CoA, and consequently, to fatty acid and cholesterol. Arginine, histidine, ornithine and proline enter the TCA cycle indirectly through glutamic acid at the α-keto glutarate stage.

By reversal of the reaction by which amino acids enter the TCA cycle, synthesis of non-essential amino acids by amination occurs in the liver. For example, pyruvic acid, OAA and α-keto glutaric acid are aminated to alanine, aspartic and glutamic acid respectively. Again, when the liver is flooded with a mixture of amino acids, a greater proportion is deaminated and oxidized, and thereby the amount of pyruvic acid, OAA and α-keto glutaric acid are increased. For synthesis of tissue protein from amino acids, ATP is needed. This demand is met by increased rates of TCA cycle reactions. Thus, high amino acid concentrations lead to increased TCA cycle function with increased ATP synthesis. Under certain pathogenic conditions, when excessive tissue protein breaks down to meet the energy requirements, oxidation of fatty acid (acetyl CoA) increases with an increase in TCA cycle activities. For efficient oxidation of acetic acid, adequate amounts of catalytic OAA are provided. Normally, this comes primarily from pyruvic acid in the liver, the oxidative deamination of aspartate, and from α-keto glutaric acid formed upon entrance of other amino acids into the cycle.

During starvation, breakdown of tissue proteins prevails and energy is primarily derived from oxidation of fatty acid from the TCA cycle. Of course, the severity is less in starvation, as keto acids formed in the deamination process can be efficiently oxidized to yield energy.

OAA and other cycle metabolites from amino acids occur largely in the liver and then pass on to the tissues via blood, where they are used by TCA oxidation.

The branching points of intermediary metabolites of fat, protein and carbohydrate are shown in figure 23.

K. The Present State of Carbohydrate Metabolism in Uremia

The vast literature covering the encyclopaedic works done on the patho-physiological effects of uremia on carbohydrate metabolism have been reviewed first by O'Brien et al. in 1965 (248), Wills in 1968 (21), Merrill et al. in 1970 (249), and most recently by Defronzo et al. in 1973 (250) after the background materials were reviewed and published by many workers (20,104,251,252,253).

There is no coincidence to suggest that the kidney plays a preliminary role in control mechanisms for glucose and lipid homeostasis, as the plasma glucose concentration is normally maintained at a value well below the renal threshold for glucose (between 125 to 160 mg% in man). Under normal conditions, virtually all filtered glucose is reabsorbed from the tubules by an active transport process.

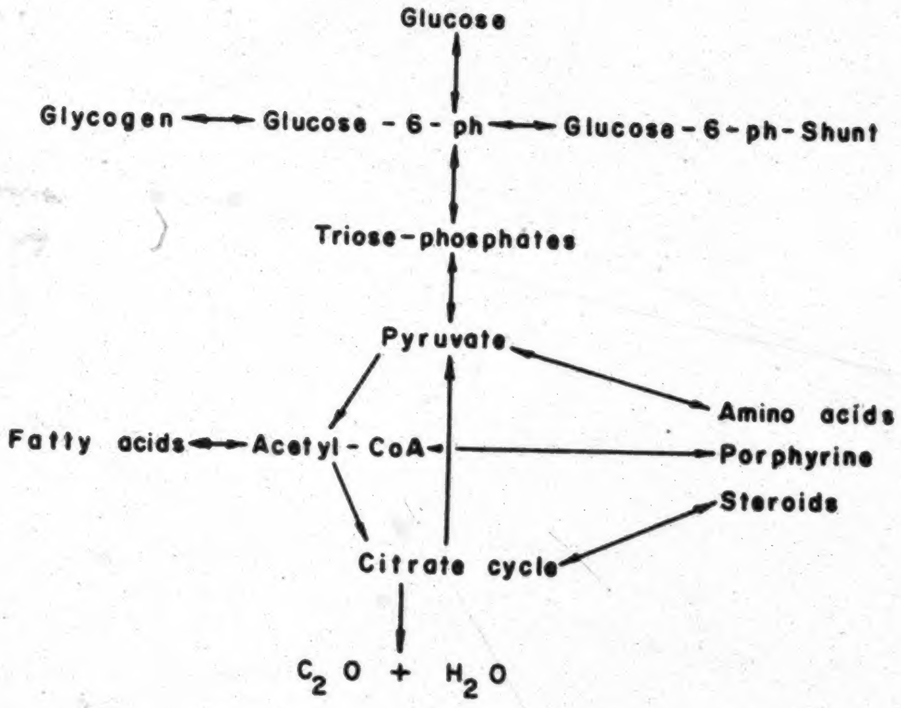

Glucose
Glycogen
Glucose - 6 - ph
Glucose - 6 - ph - Shunt
Triose - phosphates
Pyruvate
Amino acids
Fatty acids
Acetyl - CoA
Porphyrine
Steroids
Citrate cycle
C_2 O + H_2 O

Fig. 23. Branching points of the intermediary metabolism.

Of course, the kidneys play an important role in metabolism and degradation of insulin; they are secondary only to the liver in the regulation of plasma insulin concentration (254).

The glucose tolerance (i.e. capacity to dispose of administered glucose) is abnormal in renal failure but fasting hyperglycemia is rare (255,256,257).

Uremia is known to interfere with carbohydrate metabolism in two apparently opposite directions. In non-diabetic but uremic patients glucose intolerance is often seen (258,259), while a diabetic when uremic often shows a decreased insulin requirement (260,261). Balestri _et_ _al_. (264) found that in _in_ _vitro_ experiments, urea and creatinine inhibit the uptake of glucose by isolated rat diaphragm and by normal human erythrocytes. They also observed that artificial elevation of blood urea or creatinine causes carbohydrate intolerance in human subjects, but that methyl guanidine has no effect on the glucose tolerance of their subjects. Quite in contrast were the findings of the other workers (262,263,57), who showed in both _in_ _vivo_ and _in_ _vitro_ experiments that the guanidine, methyl guanidine and guanidinoacetic acid retained in CRF exert hypoglycemic effects quite similar to those of the biguanide oral antidiabetic agents.

Impaired carbohydrate tolerance in the whole organism together with peripheral insulin unresponsiveness (impaired glucose uptake) has been suggested by Cohen _et al_. (184) to be due to inhibition of ADP induced Platelet Factor 3 deficiency in uremics (184). The Platelet Factor 3 deficiency, implicated in uremic bleeding, bears a correlation with GSA levels before and after dialysis treatment.

A GSA induced defect in the phosphorylating coenzyme is due to its inhibitory effect on ADP, on Platelet Factor 3 release, and upon hexokinase or other related enzymes involved in the cellular uptake of glucose. Impairment of both glycogenesis and glycogenolysis occurs in the liver, a principal site of this defect in carbohydrate metabolism, a site in which the requirement for ATP is greatly enhanced by the increased requirement for urea synthesis from ammonia and deamination reactions. Hutchings _et al_. (265) underscored the latter effect by inducing carbohydrate tolerance by the addition of urea to the dialysate during dialysis; impaired carbohydrate tolerance in CRF is otherwise usually improved by hemodialysis (267,268) or diuresis (266). Hutchings _et al_. (265) and Saglid (266) have differentiated uremic carbohydrate intolerance from the effects of starvation, infection, inactivity, electrolyte, and hereditary factors, and showed it to be independent of qualitative and quantitative defects in insulin or of circulating antagonists. Hypoglycemia is common after dialysis due to endogenous insulin secreted in response to hyperglycemia prior to dialysis (351). Several theories have been proposed to account for the abnormal carbohydrate tolerance seen in CRF:

i. defects in insulin synthesis or release.
ii. the presence of circulating insulin antagonists.
iii. defects in peripheral glucose utilization.
iv. defects in hepatic glycogen storage and/or glycogenolysis.
v. other factors.

A summary of these theories is present in the following paragraphs.

1. Insulin Synthesis and Release

Conn (269) suggested that the normal rapid release of insulin from the pancreas in response to a glucose load involves a specific concentration of potassium within the β-cells and also extracellular fluid concentration of sodium and calcium (270,271). Therefore, homeostatic alterations in any of these ions may be involved in abnormal carbohydrate tolerance in uremia.

Gorden et al. (272) observed that 10% of the insulin released after oral glucose stimulus is a single chain precursor of insulin with less biological activity; levels of this 'big insulin', are elevated in the uremic on glucose challenge.

Following I.V. glucose stimulus in the uremic, the plasma insulin early response shows conflicting values; some show normal levels (273), and in others there is an increase (265) or a decrease (267). Late insulin levels have been reported to be elevated (274). Prolonged half-life of insulin is known to occur in bilateral but not in unilateral nephrectomy in humans (254). (The basal plasma arterial insulin level is 15 μU/ml). The recent review by Rubenstein _et al_. (254) also reported inhibition of some other insulin degrading organ systems to occur in uremia. This inhibition could be corrected by dialysis. Spitz _et al_. (265,275) speculated that the hyperinsulinemia seen in uremia after glucose administration and the prolonged hypoglycemia following exogenous insulin and tolbutamide (66) might result from a decreased ability of the liver to degrade insulin; this was supproted by Wildberger _et al_. (276) in that the nephrotic rat liver extracts inactivated less insulin than controls (normally liver degrades less than 50% of the insulin released in portal circulation).

The discrepancies in the response of insulin to glucose stimulation (which in the normal can show hyperinsulinemia immediately after glucose administration) might be due to interplay between insulin secretion and peripheral antagonism (250). Sagild _et al_. (277) showed that the glucose utilization index, K, decreased with depletion of potassium (as little as 200 to 569 mEq).

Milner et al. and others showed the importance of the potassium ion in the pancreatic release of insulin (281,270, 271). Therefore, all suggested that a possible cause of uremic glucose intolerance might be diminished pancreatic insulin release secondary to hypokalemia, but that this cannot be the only factor. Spergel et al. (278) observed that oral potassium treatment improved glucose tolerance but did not completely return glucose tolerance to normal.

2. The Presence of Circulating Insulin Antagonists

Peripheral insulin antagonism has now been well documented by several evidences:

(a). Forearm perfusion studies of Westervelt (279,280) showed that during the basal period, glucose uptake in uremia is similar to that in controls. However, 45 minutes after intra-arterial infusion of insulin the forearm glucose uptake in the uremic is only 25% of that in the control; lactate production on a molar basis of glucose utilized was the same in uremics and controls. Phosphorus uptake also decreased. They did not distinguish between this peripheral insulin antagonism, a defect in insulin mediated cell membrane transport of glucose, or a defect in glucose phosphorylation. However, once glucose enters the cell and becomes phosphorylated, the Embden-Myerhof pathway seems to be intact.

(b) The fasting level of glucose is normal but the insulin level is higher as shown by many workers (265,273,275,282).

(c) There is delayed and diminished fall of blood glucose after exogenous insulin administration (267,283,275) and

(d) Tolbutamide administration (66, 267,275). Mechanisms postulated are:

i. circulating antagonism is due to elevated fasting plasma growth hormone in most of the uremics; (252,282) the levels of which are known to be inversely correlated to serum albumin; this suggests abnormal growth hormone response to be a reflection of protein malnutrition, secondary to renal failure.

ii. cellular antagonism: accumulation of uremic toxins antagonize cells via non-insulin dependent mechanisms, probably by inhibiting enzymes such as PFK

(284) or uncoupling oxidative phosphorylation in liver mitochondria with a resultant decrease in ATP levels (284).

iii. metabolic end products (265,275).

iv. acidosis.

Twenty-five percent of the patients receiving kidney transplants were reported to develop impaired carbohydrate tolerance as a result of large doses of glucocorticoids. Diet and tolbutamide cure this effect. The effect also subsides when the prednisone dose is lowered (285).

It has been shown that the abnormal glucose tolerance associated with CRF can be corrected with adequate hemodialysis (267,268,274), implicating the dialysable low molecular weight substances in causing peripheral insensitivity to insulin.

The high plasma insulin concentrations in patients with CRF are associated with a markedly raised urinary insulin excretion and insulin clearance (286) which excludes a defect in either the synthesis or release of insulin as a major glucose intolerance in uremia. A reduction in glucose utilization by rat liver slices incubated in uremic sera also supports the concept of "retained serum factors" as the cause of insulin antagonism (287). Perkoff et al. (279) disproved that urea alone can be incriminated in carbohydrate intolerance in uremia.

3. Peripheral Glucose Utilization Defects

Carbohydrate intolerance is thought to be due to a primary alteration in either peripheral tissue metabolism or uptake, secondary to the metabolic changes of renal failure (279-288). Impaired phosphorylation of glucose at the cellular level, and impaired tissue glucose uptake due to competitive blockade of phosphorylating coenzymes, are proposed to be causes of impaired carbohydrate tolerance in uremia (279,280,288,66).

4. Defects in Hepatic Glycogen Storage and Glycogenolysis

Cohen _et al_. implicated the liver in abnormal carbohydrate tolerance, suggesting that after oral administration of glucose and I.V. injection of insulin, the hyperglycemia indicated the liver's inability to take up glucose. They felt that the prolonged hypoglycemic response of I.V. insulin was due to diminished hepatic glycogen stores secondary to a defect in glycogen synthesis or release (glycogenolysis) (289). In subsequent studies he observed subnormal increases in blood glucose following both glucagon and epinephrine, and a markedly abnormal galactose tolerance test in uremics (289) as well as in nephrectomized rats. He suggested that the failure of uremics to dispose of galactose (which is normally taken up by the liver) and the subnormal response to glucagon and epinephrine were due to a defect in hepatic glycogenesis (289). Luke et al. (293) found fructose tolerance in uremics despite the presence of glucose intolerance, although fructose is primarily metabolized by the liver enzyme FK; they concluded that the Embden-Meyerhof pathway below the F-1,6-DP level must be intact. Dzurik et al. (290) and Boucto et al. (291) demonstrated normal liver glycogen content in uremic patients and in acutely and chronically uremic rats. Cummings et al. (292) found that incorporation of glucose into glycogen, lipid and CO_2 by chronically uremic rat liver was normal. Dzurik et al. (287) also observed that uremic serum incubated with rat liver slices was without effect on glycogen synthesis.

5. Other Factors

An absolute increase in hepatic gluconeogenesis or an inability of the liver to decrease glucose production in response to insulin may be other causes for glucose intolerance in uremia. These possibilities have not been explored except for the observation that concentrations of plasma amino acids including alanine, the chief gluconeogenic substrate (294,295), diminished in uremic patients (157).

Hampers et al. (267) and Lowrie (273) found that in spite of good nutrition, patients on chronic dialysis showed persistent carbohydrate intolerance.

L. The Treatment of Chronic Renal Failure

1. Hemodialysis

The toxemic effects of toxic metabolites and amino acids in the etiology of the uremic syndrome remain unclear, but their removal by hemodialysis and peritoneal dialysis are continually done (297-300). Renner et al. (301) showed that unless these substances are removed by dialysis, they inhibit enzymes, diminish utilization of pyruvate, β-hydroxybutyrate and acetoacetate by tissue slices, and reduce the rate of formation of glucose from pyruvate (probably due to a reduction in ATP synthesis). Uric acid in CRF led to infrequent occurrence of secondary gout due to the difference in uric acid metabolism as compared to normal subjects (3,303). This may be due to uricolysis (304), that is, the extrarenal elimination of uric acid catalyzed by intestinal bacteria.

2. Protein Diets in Renal Failure

It has been recently shown that with selective low protein diets containing the "minimum safe" quantity of essential amino acids, uremic symptoms can be controlled and yet the nitrogen balance maintained (307-311). The principles of low protein diets in uremia, avoidance of deficiency in calories and essential amino acids have been reviewed (312-314,184). One such special diet is the Giordan-Giovannetti Diet. In 1963 Giordano (307) and in 1964 Maggiore (308) reported a diet adequate in amino acids but low in protein content (1/3 of a g/Kg body weight: one egg, 1/2 cup of milk) which supplies more than 1/2 the daily protein intake and the minimum daily required amino acid. This diet showed that more urea nitrogen found its way back into protein and less urea was produced, and the BUN fell by 50%. Since then others adapted this diet to suit their national tastes (309-311).

The caloric intake should be 30 to 35 cal. per Kg to utilize protein the form of fruit, beverages, sugar, butter, oil, etc.

The patients surviving on this diet develop side effects including hyperkalemia, ventricular irregularities, and convulsions. Therefore, potassium from vegetables should be removed by soaking in water. Then, of course, water soluble vitamins have to be supplied.

3. Homologous Kidney Transplantation

This is the only permanent solution to the uremic problem.

M. Summary of the Metabolic Disturbances in CRF

The disturbances in the internal environment in CRF is complex and multifactorial, as pictured in figure 24. The retained metabolites and organic compounds trigger metabolic derangements acting at subcellular levels as enzyme inhibitors. The retention of urea affects the sodium and water balance by promoting an osmotic diuresis; retained metabolic products affect changes in acid-base

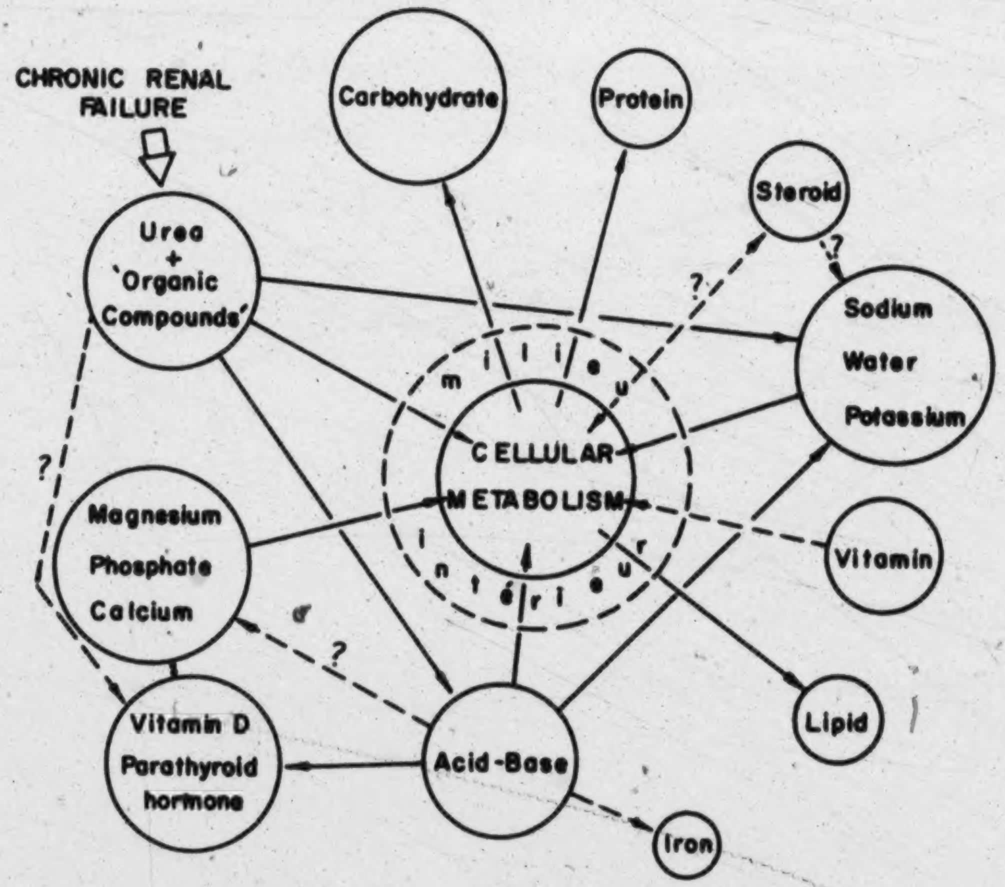

CHRONIC RENAL FAILURE
Carbohydrate
Protein
Steroid
Urea + 'Organic Compounds'
?
Sodium
Water
Potassium
milieu intérieur
CELLULAR METABOLISM
Magnesium
Phosphate
Calcium
Vitamin
Vitamin D
Parathyroid hormone
Acid-Base
Lipid
Iron

Fig. 24. Diagram illustrating the changes in the internal environment in chronic renal failure (J. Clin. Path. 21, 552, (1968)).

balance. Also, these, either directly or by altering PTH or Vitamin D sensitivity, affect the Ca, P and Mg balance which consequently contributes directly to the alterations in cellular metabolism of protein, lipid, electrolyte, carbohydrate and steroid metabolism. In the alterations of carbohydrate metabolism, insulin antagonists seem to play a dominant role.

The biochemical mechanisms involved in the uremic syndrome of CRF are yet to be elucidated.

CHAPTER III

EXPERIMENTAL

A. Animal Management

1. Sub-Total Nephrectomy

Sub-total and contralateral nephrectomy in male Wistar rats was performed first by 70 to 80 percent nephrectomy of the left kidney followed by complete removal of the right kidney one week later, under methoxyflurane anesthesia (Penthrane, Abbott Laboratories Ltd., Montreal, Quebec), according to the method of Kessner and Epstein with the modification of McCance and Morrison (315,316). Hemostasis was created by oversewing the cut ends of the excised kidney with "Gel-foam" (The Upjohn Co., Kalamazoo, Michigan). Care was taken not to disturb the adrenal glands during surgery. The mortality rate was less than one per cent. Chronic uremia was ellicited by long-term maintenance (up to 19 weeks or more of sub-total and contralateral nephrectomized rats. Sham-operated (SO) rats, made by opening their abdomen and revolving both kidneys, one after another, and replacing them in the abdomen, were used as controls. Animals were considered to be uremic (U) if they had persistant elevation of blood urea nitrogen above 30 to 35 mg per cent. The method used was that of Fawcett _et al_. (320).

2. Collection of Blood and Tissue Samples

One ml of blood was collected from a stab incision at the ventral position of the base of the tail (317) at fort-nightly intervals. The blood was collected into a capillary tube for serum and in a heparinized capillary tube for plasma, for later preparation and analysis.

Three to four milliliters of blood were sampled in a heparinized tube from the periorbital sinus for amino acids study (317).

Terminal blood samples of 8 to 10 ml were collected from the aorta of anesthetized rats by a heparinized syringe.

The pectoral muscle, liver, kidney and adipose tissue were excised from rats exsanguinated under ether anesthesia for use in enzyme, glycogen, lipid and other studies.

3. Diet Restrictions

(See Appendix F)

The restricted diet (RD) control animals were allowed only 23 g of food daily; this amount provided a daily food consumption equivalent to that of the uremic rats and represented approximately 70 to 80 percent of the food intake of healthy rats of the same age.

4. Maintenance

All rats were singly caged and maintained in a constant temperature environment (22±2°C) with 12 hours of artificial light per 24 hours. Standard Teklad mouse-rat diet (Teklad Inc., Winfield, Iowa) and water were available *ad lib*. The restricted diet (RD) control animals were also allowed water *ad lib*.

Rats were permanently identified by ear punching.

B. Methods and Materials

All reagents and chemicals used were analytical or biochemical grade.

1. Radiochemical Purity Assay (318,319)

Radiochemical purity of D-glucose-(U)-^{14}C and glucose-^{14}C-1 was checked by using:

(a) Paper chromatography in:

i. n-butanol:ethanol:water (52:33:45)

ii. n-butanol:pyridine:water (1:1:1)

iii. ethyl acetate:acetic acid:water (9:2:2) containing two percent phenylboronic acid

(b) Thin layer chromatography in:

i. n-butanol:ethanol:H_2O (50:32:18)

ii. isopropanol:H_2O (160:40)

(c) Autoradiochromatography

Autoradiochromatographs were made by exposing medical no-screen X-ray film (Picker Nuclear, White Plains, N.Y.) to the developed thin layer chromatographic plates of ^{14}C-glucose for 14 days in the dark (see fig. 25). The radiochemical purity of the ^{14}C-glucose was determined to be at least 98 percent pure, by liquid scintillation counting of radioactive spots on the thin layer chromatograms.

2. Radioactivity Measurement

Radioactivity measurements were made by liquid scintillation counting in an appropriate fluor. Counting efficiency was determined by using an internal standard (^{14}C-Toluene, sp. activity 4.17×10^5 dpm/ml, New England Nuclear, Boston, Mass.) using a Picker Liquimat 220 (Picker Instruments) liquid scintillation spectrometer, (Picker Nuclear, White Plains, N.Y.). Radioactive tissues were first combusted to $^{14}CO_2$ using a Packard Model 306 oxidizer (Packard Instruments, Downers Grove, Illinois). Packard Permafluor^R and Carb-o-sorb^R scintillation solvents were used. Recovery tests were frequently performed by burning n-Hexadecane-1-^{14}C (1.10 µCi/g) and ^{14}C-toluene (activity 4.17×10^5 dpm/ml). This reference standard and the counting efficiency correction were used to calculate activity in dpm.

3. Biochemical Blood Analysis

(a) BUN

Principle

Plasma is incubated with buffered urease for quantitative conversion of urea to ammonia. Ammonia then is

Fig. 25. Auto-radiochromatography of ^{14}C-glucose used in the experiments.

measured by a modification of the Berthelot (321) reaction in which a blue color is formed in the presence of sodium phenate and hypochlorite. The intensity of the blue color (read at 540 nm) is proportional to the ammonia concentration. The adult normal range (fasting) for this method is from 8 to 20 mg N/100 ml plasma.

(b) Serum glutamic oxaloacetic transaminase (SGOT) was determined by the ESKALAB method, (Smith, Kline and French Laboratories, 440 Page Mill Rd., Palo Alto, California) using ESKALAB SGOT tablets, disposable cuvette assemblies and the spectrophotometer Alpha at a wave length of 340 nm (322). The stepwise procedure specified by the ESKALAB (322) was used.

Principle

SGOT accelerates the simultaneous transformations of α-ketoglutaric acid to glutamic acid and aspartic acid to oxaloacetic acid by transfer of the α-amino group of the aspartate to the α-ketoglutarate. The rate of formation of oxaloacetic acid is proportional to the enzyme activity present. To quantitate this first reaction, the oxaloacetic acid formed is further converted to malic acid by the enzyme MDH. For each mole of oxaloacetic acid reduced by MDH an equimolar amount of $NADH_2$ is oxidized to NAD^+. The rate of $NADH_2$ oxidation is directly related to the amount of SGOT in the sample. The $NADH_2$ oxidation is accompanied by a decrease in absorbance at 340 nm and the rate of absorbance change is proportional to SGOT activity. The reaction at pH 7.5±0.1 at 37°C is as follows:

$$\text{L-aspartate} + \alpha\text{-ketoglutarate} \xrightarrow{\text{SGOT}}$$

$$\text{L-glutarate} + \text{oxaloacetate}$$

$$\text{oxaloacetate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{MDH}} \text{L-malate} + \text{NAD}^+$$

ESKALAB reagent tablets contain all materials necessary for the assay of this enzyme by the method of Karmen (323) as modified by Henry *et al*. (324).

For a 10 µℓ serum sample, the initial and final absorbance "A" should always be greater than 0.60 and 0.400, respectively. The results were expressed in IU (µmoles/min/litre of serum). Normal values for this method using ESKALAB control (normal) serum was between 16 to 19 IU at 37°C in our laboratory. Usual normal human is 9 to 40 IU.

(c) Creatine Phosphokinase (CPK)

CPK was determined in serum samples (10 µℓ) using the standard "ESKALAB" method and reagent tablets (322) and spectrophotometer at 340 nm with a preincubation time of ten minutes and incubation (at 37°C) time of five minutes.

Principle of Test

CPK accelerates the transfer of phosphate from creatine phosphate to ADP, forming creatine and ATP.

The ATP formed in the CPK mediated reaction is used to produce G-6-P from glucose included in this reaction mixture. This reaction is catalyzed by the enzyme HK, incorporated into the reagent tablet. As G-6-P is formed ADP is generated keeping its concentration at a constant level. The G-6-P formed by the HK reaction is then oxidized by the enzyme G-6-PDH with simultaneous reduction of NADP. The reduction of NADP to $NADPH_2$ is followed spectrophotometrically by observing the resulting increase in absorbance at 340 nm. For each mole of phosphate transferred by the CPK a mole of $NADPH_2$ is formed. Thus, the rate of ΔA is directly proportional to the CPK activity present in the sample.

AMP is included in the reagent to inhibit myokinase (325). The glutathione, a sulfhydryl compound acts as an enzyme stabilizer (326).

ESKALAB reagent tablets for serum creatine phosphokinase contain all the materials necessary for assay of this enzyme by a modification of the method of Oliver (325,326,327).

Dilution of serum because of high CPK activity should be used with caution as activity in some cases changes at certain dilutions.

The assay should be repeated if the initial absorbance reading exceeds 0.7 A units and the difference between readings (absorbance at 10 min. minus absorbance at 15 minutes) is greater than 0.300 A units.

The normal range in serum in our laboratory was between 76 to 115 IU at 37°C.

(d) Alkaline Phosphatase

Alkaline phosphatase was determined in plasma (heparinized tubes were used to collect blood) using the ESKALAB reagent tablets and spectrophotometer Alpha (322) at a temperature of 37°C. The sample size used was 10 $\mu\ell$ with a preincubation time of 5 minutes and an incubation time of 10 minutes. Photometric analysis was done at a wave-length of 415 nm.

Principle

In a basic medium, phosphate mono esters are hydrolyzed by alkaline phosphatase to give the corresponding alcohols and inorganic phosphate. Using p-nitrophenyl phosphate as substrate, alkaline phosphatase catalyzes the hydrolytic reaction and Mg^{++} activates it. The reaction product, p-nitrophenol has a much higher molar absorptivity (ε) at 415 nm, than the substrate, p-nitrophenyl phosphate. The reaction is followed by measuring the increase in absorbance at 415 nm:

$$\text{p-nitrophenyl phosphate} + H_2O \xrightarrow[\text{phosphatase } Mg^{++}]{\text{Alkaline}}$$

$$\text{p-nitrophenyl} + \text{phosphoric acid}$$

The rate of hydrolysis, not the absolute product, is of interest. Therefore, two absorbance readings are made on each sample with a designated interval between readings. The activity of the alkaline phosphatase is directly related to the change in absorbance per unit time. ESKALAB reagent tablets contain all the reagents necessary for the determination of alkaline phosphatase with a modification of the original method of Bessey _et al_. (328).

(e) Hematocrit (H_{ct})

The volume of erythrocytes expressed as a percentage or ratio of the volume of whole blood was measured by using microhematocrit capillary tubes filled with blood to the black line. The opposite end of the tubes were plugged with Seal-ease, (Clay Adams, Parsippany, N.J.), and the tubes were centrifuged for ten minutes at 7,500 rpm (329). Potential sources or error were:

i. improper mixing of blood

ii. inadequate centrifugation as to duration and speed

iii. improper reading of the level of cells and plasma and inclusion of the buffy coat as part of the erythrocyte volume.

Normal H_{ct} values for rats are between 0.35 and 0.45.

(f) Glucose

Plasma and urinary glucose concentrations were measured using the method of ESKALAB and "ESKALAB" reagent tablets. Determination of glucose was made spectrophotometrically at 340 nm by the use of the coupled enzyme reaction of HK and G-6-PDH, a modification of the original method of Barthelmai and Czok (330). Ten microliters of the plasma and/or urine sample were used with an incubation time of five minutes and diluent volume of 1.5 ml per tablet at room temperature (if the level was not anticipated to be higher than 300 mg%; if so then 5 μℓ samples were used).

A plasma blank in physiological saline was run for every sample.

Principle

D-glucose is phosphorylated by ATP in the presence of HK, giving G-6-P and ADP. The reaction is activated by Mg^{++} ions. The G-6-P is then oxidized by the enzyme G-6-PDH with concurrent reduction of NADP yielding 6-phosphogluconic acid and reduced NADP. By measuring the amount of $NADPH_2$ formed in this reaction by observing the resulting increase in absorbance at 340 nm, the amount of glucose phosphorylated by the HK is estimated. The reaction being stoichiometric proceeds quantitatively.

$$\text{Glucose} + \text{ATP} \xrightarrow[Mg^{++}]{HK} \text{G-6-P} + \text{ADP}$$

$$\text{G-6-P} + \text{NADP} \xrightarrow{\text{G-6-PDH}} \text{6-phosphogluconic acid} + NADPH_2$$

Normal values in ESKALAB control serum using F = 463 (See Appendix D) with this method were 72 to 92 mg/100 ml for our laboratory.

Urinary glucose estimations were conducted in the urine collected over a period of 24, 48 and 72 hours, in rats housed in a metabolic cage designed for collection of urine. All of these rats had experienced 18 to 19 weeks of uremia. pH, glucose, and protein in the urine were checked using Combistix (Ames Co., Rexdale, Ontario); glucose was measured using the ESKALAB method (330) and a pH meter was used for accurate pH determination. The total volume of urine collected over 24 hours, 48 hours, and 72 hours was measured for three groups of rats each having one each of SO, U, and RD rats.

4. *In Vivo* Oxidation of ^{14}C-Glucose

Glucose metabolism studies were conducted in the four to six hour post-absorptive state. D-glucose (-U-^{14}C and -1-^{14}C-labelled, ICN Corporation, Irvine, California) was administered by I.P. injection of aqueous solutions. The radioactive glucose was so diluted with water as to give concentrations of one or two µCi/0.1 ml of solution. Immediately after ^{14}C-glucose administration, the rats were placed in respiratory and metabolic chambers (models CR 350 or CR 550) after conditioning the animals in restrainers (BR350 or BR550) (see models from Nuclear Associates, Inc., Westbury, N.Y.).

The expired air was passed through 3 ml of ethanolamine:methyl cellusolve (1:2) mixture to trap $^{14}CO_2$.

Fresh trapping solutions were provided every 10 minutes for a period of 3.5 to 4 hours and then for 30 minute periods over 24 hours. Complete trapping of $^{14}CO_2$ was monitored by passing the effluent gases through a trap containing ten percent sodium hydroxide. The solution containing the trapped $^{14}CO_2$ was then diluted by adding 15 ml of methyl cellusolve and toluene (1:2) containing PPO (6 g/liter). A safety trap containing sodium hydroxide solution was connected in series to the ethanolamine trap and a flow meter. The meter was used to regulate the flow of air through the respiratory chamber.

5. Isolation and Structure Determination of Glycogen

(a) Isolation

Isolation of glycogen from the liver was made according to the method of Bloom et al. (331). The liver was cut into small pieces in the cold, and mixed with one volume of 10 percent TCA and 0.5 g of washed sand per 8 g, and a paste was made using a cold mortar and pestle. The paste was centrifuged for ten minutes at 2,500 rpm. The pellet was re-extracted with one volume of 5 percent TCA, allowed to stand at 25°C for 5 minutes and re-centrifuged at 2,500 rpm for 10 minutes.

Both supernatants were then combined and the glycogen was precipitated by adding two volumes of 95 percent ethanol with constant stirring. The mixture was then allowed to stand to flocculate the precipitate; if no precipitation occurred, a pinch of sodium chloride was added, and the solution was warmed and mixed gently until the precipitate formed. In most of the experiments no addition of salt was required.

The precipitate was collected by centrifugation for 5 minutes at 2,500 rpm. The supernatant was then decanted and the precipitate was dissolved in 5 ml of water and reprecipitated with two volumes of 95 per-cent ethanol.

The glycogen was isolated by centrifugation or by suction filtration and washed sequentially with ethanol and then ether. It was dried in vacuo until constant weight was reached.

(b) Structure Determination
(See Figure 30)

Pooled glycogen obtained from either five SO rats or three uremic rats was dissolved in water and reprecipitated with two volumes of 95 percent ethanol as described above. These glycogen samples were used for the determination of the structure and molecular weight. The samples were obtained four and one-half weeks after nephrectomy.

Structural analysis, by chemical end-group assay, was made in terms of:

i. the number of moles of glucose residue per g of glycogen by periodate oxidation and

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ii. the number of non-reducing ends in glycogen, by periodate oxidation in presence of ethylene glycol (233,234,332-337).

iii. the number of reducing ends in glycogen, using glucose as a standard and measuring colorimetrically the reduction of 3,5-dinitrosalicylate (338).

iv. the number of segments per mole, number of glucose residues per segment, average number of branching points (1,6-bonds), and the molecular weight.

i. Determination of Glucose Residues Per G of Glycogen Principle

Principle: The number of moles of periodate (IO_4^-) ≡ the number of moles of glucose residue since one mole of IO_4^- attacks one glucose residue only. The periodate consumption by glycogen solution (0.5 to 0.6%) is determined after titration with N/10 thiosulfate followed by correction for the blank. Four moles of thiosulfate reacts with two moles of I_2 generated by two moles of $IO_4^- \rightarrow IO_3^-$ (one mole IO_4^- ≡ two moles $Na_2S_2O_3$).

Periodate Oxidation

Two samples containing glycogen solution (0.5 to 0.6%) and 0.2 volumes of 0.5 M $NaIO_4$ were incubated in triplicate for each of the uremic and control samples, respectively for two hours, and for seven days at room temperature in the dark. At the end of the periods, two ml aliquots were withdrawn from the respective samples, as well as from reagent blank which had been treated similarly but contained water in place of glycogen solution. To each of these two ml aliquots were added 5 ml 1N H_2SO_4 and 5 ml 20% KI. The iodine liberated was titrated with 0.1 N $Na_2S_2O_3$ using starch indicator. Corrections were made for the reagent blank.

Mechanism

Periodate oxidation of carbohydrate follows a specific and stoichiometric course. Periodate selectively cleaves a molecule between adjacent carbon atoms which have any combination of free hydroxyls, aldehydes, ketones or primary amine groups. Each oxidation is accompanied by a reduction of one IO_4^- (periodate) to IO_3^- (iodate). Free hydroxyls are oxidized to aldehydes but if cleavage occurs on both sides of the carbon atom, then one formic acid is produced. If, however, the periodate reactive group of either of two adjacent carbon atoms are tied up in another bond, as in a glycoside, no oxidation or cleavage occurs at that particular carbon-carbon bond. The number of moles of periodate consumed per g of a sample is determined by conversion (in acid with an excess of iodide) of both the unreacted IO_4^- and IO_3^- formed by the conversion to I_2:

$$8\,H^+ + IO_4^- + 7I^- \xrightarrow{H^+} 4I_2 + 4H_2O \quad \text{(Blank)}$$

$$6\,H^+ + IO_3^- + 5I^- \xrightarrow{H^+} 3I_2 + 3H_2O \quad \text{(Sample)}$$

IO_4^- and IO_3^- give different amounts of I_2 when quenched (reduced) with acidic iodine and the difference in titration value between the sample and a water blank is a measure of IO_4^- which has been reduced to IO_3^- and hence number of carbon-carbon bonds cleaved.

The I_2 formed is determined by titration with thiosulfate using starch-iodine complex as the indicator.

$$I_2 + 2\ Na_2S_2O_3 \longrightarrow 2\ NaI + Na_2S_4O_6 \quad \text{(tetrathionate)}$$

ii. Determination of the Number of Non-Reducing Ends Principle

The glucose residues at the non-reducing ends have three instead of two free vicinal hydroxyls which leads to the production of formic acid upon periodate oxidation. Because of this branched, fan-like structure of the glycogen, only the terminal reducing end and all of the non-reducing ends yield formic acid on periodate oxidation, but the amount of formic acid formed from the single reducing end is insignificant compared to that found from the many terminal non-reducing sugar residues.

In absence of ethylene glycol periodate thus attacks both non-reducing and terminal reducing glucose residues giving formic acid and dialdehydes, but in the presence of ethylene glycol, cleavage occurs only at non-reducing ends. Ethylene glycol also destroys excess periodate.

The number of non-reducing ends may be measured by titration of the formic acid produced with diluted alkali and the number of molecules of formic acid formed would be approximately equal to the number of non-reducing ends of glycogen.

Procedure

To 10 ml periodate reaction mixture was added 0.25 ml ethylene glycol. The solution was mixed well and allowed to stand at 25°C for 10 minutes. The formic acid liberated was titrated with 0.05 N sodium hydroxide, using a phenolphthalein indicator. A correction for blank (using water in place of glycogen solution) was made.

iii. Determination of Reducing Ends

Principle

The reducing end has an aldo group which can reduce an oxidizing agent, 3,5-dinitro salicyclic acid monosodium salt (Eastman Kodak Co., Rochester, N.Y., U.S.A.) and can be measured by means of this reduction. Each reducing end should have the same reducing power as one molecule of glucose.

Procedure

The number of reducing ends were determined by using glucose (1 mg/ml) as a standard and measuring colorimetrically, the reduction of 1.5 percent, 3.5-dinitrosalicyclic acid monosodium salt in distilled water.

A series of standard glucose solutions containing 50, 100, 200, 300, and 450 micrograms of glucose were used to prepare the standard glucose curve. The unknown glycogen solutions for uremic and control (50 mg/ml) samples were taken, each in duplicate, (0.5 ml for uremic and 1.0 ml for the control). A reagent blank was prepared using 1.0 ml water and all the tubes were adjusted to the same volume of 1.0 ml as the reagent blank with addition of water. Then 0.5 ml of 1.5 per cent 3,5-dinitro salicyclic acid monosodium salt was added to each of the ten tubes, which were then heated for 5 minutes in a boiling water bath. The samples were removed and cooled in cold water and the contents of each tube was diluted to 10 ml with distilled water. Optical density of the standard and the samples, at 540 nm using Beckman DU Spectrophotometer (Beckman Instruments, Inc., Fullerton, California), was read against the reagent blank.

Data was plotted to obtain a graph of OD as a function of μg of glucose (see fig. 26). From the curve the number of reducing ends in terms of glucose in the glycogen were interpolated.

6. Autolysis of Glycogen

Autolysis of glycogen obtained from U, SO and RD rats was carried out by exposing excised liver at room temperature for 30 minutes and one and one-half hours, respectively. At the end of each period glycogen isolation

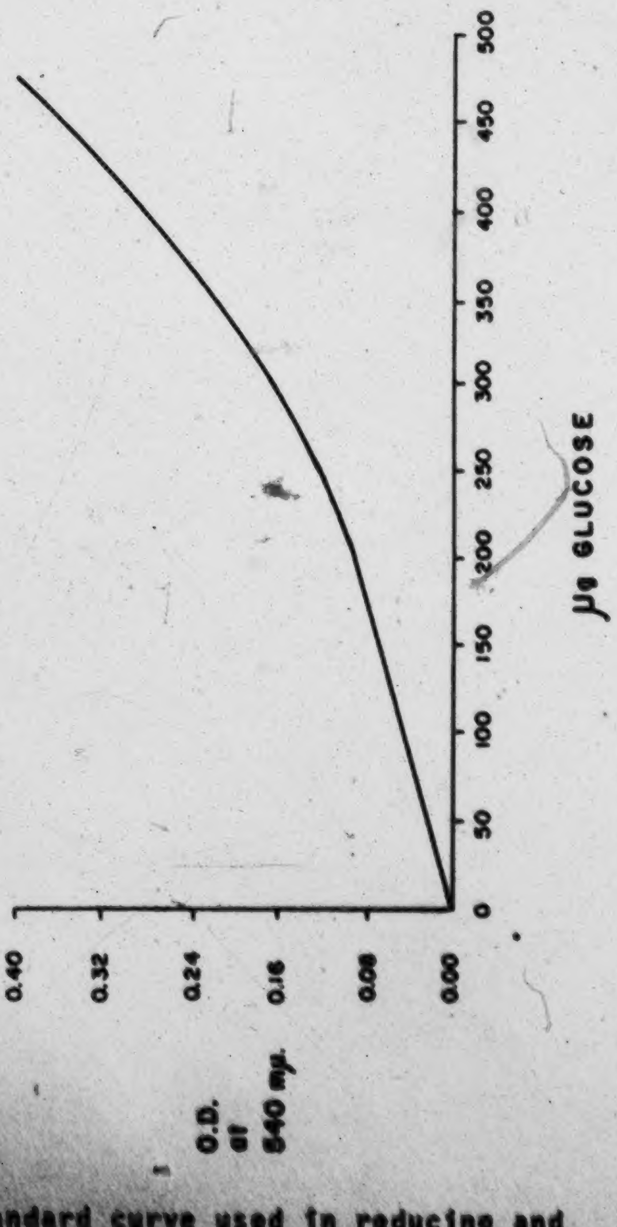


Fig. 26. Glucose standard curve used in reducing and determination.

and quantitation were made and compared with those obtained from the identical liver samples maintained, respectively at 0 to 4°C.

7. Assay of Glycogen Cycle Enzymes in Muscle and Liver

(a) Amylo-1,6-glucosidase

Determination of amylo-1,6-glucosidase was made using the method of Hers (339). Both liver and muscle homogenenates of U, SO and RD rats were used.

Principle

This method is based on the observation of Larner and Schliselfeld (340) that the hydrolysis of the limit dextrin is reversible to a slight extent.

The unit of activity is defined as the amount of enzyme incorporating 0.1 per cent of the counts added as glucose in one hour. The incubation is carried out at 37°C for one hour.

$$^{*}G + G(n) \xrightarrow[\text{(Amylo-1,6-glucosidase)}]{\text{Tissue homogenate}} {}^{*}G(n+1) + H_2O$$

$^{*}G = {}^{14}C$-glucose

Composition of the Substrate Solution

Glycogen	3.12 mg
$^{*14}C$-glucose	0.60 ml ($\equiv 1.33 \times 10^8$ dpm)
Sörensen potassium-phosphate buffer (pH 7.4)	1.80 ml
Total volume	2.40 ml

The assay procedure, inhibition of the reaction, and isolation of glycogen, were according to the method of Hers (339). The isolated glycogen was dissolved in 0.5 ml water and then combusted using the Packard sample oxidizer and counted by liquid scintillation counter. The enzyme activity is expressed as total activity in μmole glucose/g tissue/hour and also in Hers units (i.e. 0.1 percent of ^{14}C-glucose added per g tissue per minute).

(b) Glycogen Synthetase "I" and "D"

The measurement of glycogen synthetase and α-glucan phosphorylase (UDP glucose:glycogen α-4 glucosyl transferase, EC 2.4.11 and α-1,4-glucan:orthophosphate glucosyltransferase, EC 2.4.1.1) activity in muscle and liver tissues of U, SO, and RD rats were carried out at 37°C using the method of Russell *et al*. (341).

Principle

Glycogen synthetase occurs in two forms, "I" active in the absence of G-6-P, and "D" only active in the presence of G-6-P. Glycogen is directly synthetized from UDPG and the reaction is catalyzed by glycogen synthetase. In addition, the "branching factor" [α-1,4-glucan: α-1,4-glucan 6-glycosyltransferase (EC 2.4.1.18)] is required to effect branching of the linear chains formed by synthetase activity as synthetase is not effective in adding branching to long, linear glucose polymer chains.

In the appropriate buffer and assay medium UDPG-glucose-^{14}C is used as substrate for reaction with the tissue homogenate. The rate of incorporation of labelled glucose into primer glycogen is measured.

$$\text{UDPG}^* + G_n \xrightarrow[\text{Synthetase and "D"}]{\text{Tissue homogenate}} {}^*G\,(n+1) + \text{UDP}$$

G_n = glycogen primer

Composition of the substrate Solution

"I"	"D"
glycogen (0.045 g)	glycogen (0.075 g)
UDPG-^{14}C (1.50 ml) (≡3.33 x 10^5 dpm)	UDPG-^{14}C (2.50 ml) (5.55 x 10^5 dpm)
Tris buffer (pH 8.2) (0.75 ml)	G-6-P (25.7 mg)
	Tris buffer (pH 8.2) (1.25 ml)
Total Volume = 2.25 ml	Total Volume = 3.75 ml

The tissue homogenate was prepared as per the procedure of Russell *et al*. (341).

For the assay, 0.1 ml of substrate was equilibrated at 37°C for 15 minutes and to this was added 0.1 ml tissue homogenate previously equilibrated for 15 minutes at 37°C. The reaction was allowed to proceed for 15 minutes. The reaction was stopped by adding one ml of an ice cold solution containing 60 mg TCA, 2 mg LiBr and 1 mg [illegible].

The isolation of glycogen followed the procedure of Russell et al. (341), using ethanol precipitation.

Measurement of Radioactivity

The isolated glycogen was dissolved in 0.5 ml water and burned in the sample oxidizer (Packard Oxidizer Model 306). For combustion the sample was placed into Combusto cones T.M. with or without Combustaid T.M. (Packard Instrument Co., Inc., Downer Grove, Illinois, U.S.A.).

(c) α-Glucan Phosphorylase "a" and "b"

Principle

Depolymerization of glycogen is catalyzed by α-glucan phosphorylase (α-1,4-glucan:orthophosphate glucosyltransferase; EC 2411). This enzyme operates readily in the reverse direction, only if sufficient G-1-P is present, to yield glycogen. This enzyme has two forms; "a" which acts independently of 5-AMP and "b" which is 5-AMP dependent (342).

$G\text{-}1\text{-}P^{14}C$ is used as a substrate in an appropriate medium for reaction with tissue homogenate, and the rate of incorporation of labelled glucose into primer glycogen is measured.

$$*G\text{-}1\text{-}P + G_n \xrightarrow[\alpha\text{-glucan phosphorylase}]{\text{Tissue homogenate}} *G(n+1) + P_i$$

COMPOSITION OF THE SUBSTRATE SOLUTION FOR

Phosphorylase "a" + "b"			Phosphorylase "a"		
Glycogen	0.066	gm	Glycogen	0.066	gm
^{14}C-G-1-P	0.15	ml	^{14}C-G-1-P	0.15	ml
	(≡6.66 x 10^6 dpm)			(≡6.66 X 10^6 dpm)	
Homobuffer (pH 6.10±0.05)	3.0	ml	Homobuffer	3.00	ml
5'-AMP-sodium	2.4	mg			
Total volume	3.15	ml	Total volume	3.15	ml

The reaction was allowed to proceed for 5 minutes at 37°C. Methods of preparation of tissue homogenate, assay and isolation of glycogen, combustion and the measurements of radioactivity were the same as those described previously for synthetase.

(d) Reagents and Chemicals Used For Glycogen Cycle Enzyme Assays

i. AMP-sodium (Sigma Chemical Co.)

ii. α-D-glucose-^{14}C(U)-1-P disodium salt (50.0 μCi, 0.077 mg; specific activity 218 mCi/mM and 0.05 mCi/2.5 ml) (New England Nuclear, Boston, Mass.)

iii. Uridine diphosphate glucose-^{14}C; specific activity 228 mCi/mM; 10 μCi/ml, (Schwarz/Mann, Orangeburg, N.Y.).

iv. U-^{14}C-glucose, specific activity 240 mCi/mM, 100 μCi/ml (Schwarz/Mann, Orangeburg, N.Y.).

v. Sodium glycerophosphate (Merck and Co., Ltd., Montreal).

vi. EDTA (Ethylenediamine tetra acetic acid) (BDH Chemical Ltd., Poole, England).

vii. NaF (sodium fluoride) (BDH Chemical Ltd., Poole, England).

viii. Mercaptoethanol (Eastman Kodak Co., Ltd., Rochester, N.Y.).

ix. Glycogen (Fischer Scientific Co., Pittsburgh, Pa.); also isolated by us in our laboratory.

x. Potassium dihydrogen phosphate (KH_2PO_4) (Fischer Scientific Co., Fairlawn, J.N.).

xi. Sodium monohydrogen phosphate (Na_2HPO_4 $2H_2O$) (Fischer Scientific Co., Fair Lawn, N.J.).

8. Assay of Glucose-6-Phosphate Dehydrogenase in Liver

Glucose-6-phosphate dehydrogenase (G-6-PDH) was assayed in 20,000 x g liver supernatant by using the stepwise procedure specified by ESKALAB (322). The reaction was carried out using ESKALAB reagent tablets and spectrophotometer Alpha with a preincubation time of 2 minutes and an incubation time of 5 minutes at 37°C. Photometric readings were taken at a wavelength of 340 nm. Ten μℓ samples were used.

Principle

G-6-PDH accelerates the oxidation of G-6-P. The reaction can be followed by observing the increase in absorbance at 340 nm caused by the formation of $NADPH_2$. This procedure will measure the combined activity of G-6-PDH and 6-phosphogluconic dehydrogenase, if any, present in liver homogenate, and the combined activity will be proportional to the G-6-PDH activity. The ESKALAB method was used assuming the 20,000 x g supernatant of liver homogenate to be equivalent to biological fluid. So far this method has been used only for specimens like blood and erythrocyte hemolysate.

$$\text{G-6-P} + \text{NADP} \xrightarrow{\text{G-6-PDH}} \text{6-phosphogluconolactone} + \text{NADPH}_2$$

Composition of ESKALAB Reagent Tablets

ESKALAB reagent tablets contain all materials necessary for the assay of this enzyme by a modification of the method of Zinkham, et al. (343).

The enzyme activity was expressed in IU/g liver/ minute at 37°C.

Preparation of Liver Homogenate

Liver tissue was homogenized in Tris buffer (pH 7.5) (2.5:1 w/v), in a Potter Elvehjem all glass homogenizer.

The homogenized tissue was centrifuged at 20,000 x g for 30 minutes using Sorvall centrifuge (Ivan Sorvall Inc., Norwalk, Conn.) at 0 to 4°C.

The clear 20,000 x g supernatant was siphoned off by Pasteur pipette and 10 lambda of clear supernatant was used for assay of G-6-PDH.

The Tris buffer used was prepared with 500 ml of 0.2 M Tris and 400 ml of 0.2 N HCl (344).

9. *In Vivo* Conversion of ^{14}C-Glucose into Glycogen, Lipids and Proteins

Conversion of ^{14}C-glucose (U) (Radiochemical Centre, Amersham, Great Britain; specific activity 250 mCi/ mM; 1 mCi/ml) to fat and protein was measured 12 hours after ip injection of the isotope during which time food and water was provided. Tissues were collected after the rats were exsanguinated under ether anesthesia.

Distribution of Total Lipid in Muscle, Liver, and Adipose Tissues

Total lipid from muscle and liver was extracted by using the method of Bligh and Dyer _et al_. (346); it was measured gravimetrically.

Procedure

Muscle: 5 g muscle was homogenized for 2 minutes with a mixture of 5 ml chloroform and 10 ml methanol. To the mixture was then added 5 ml chloroform, and after homogenizing for 30 seconds, 5 ml distilled water; blending was continued for another 30 seconds. The homogenate was filtered through Whatman No. 1 filter paper on a Coors No. 3 Büchner funnel with slight suction. The residue tissue was re-extracted along with the filter paper, with 5 ml chloroform, and the mixture was filtered through the original Büchner funnel. The homogenizer jar and residue were rinsed with a total volume of 2.5 ml chloroform. Both filtrates were combined and the alcoholic layer was removed by aspiration after noting the volume of the chloroform layer which contains the purified lipid. All or an aliquot of the chloroform layer was evaporated in a tared flask to constant dryness under nitrogen (at 40° water bath) and the lipid was estimated gravimetrically after drying the residue over phosphoric anhydride in a vacuum dessicator. For radioactivity determinations, an aliquot of the chloroform layer or a weighed dried lipid sample was combusted in the oxidizer; the sample radioactivity was then counted by LSC as described elsewhere.

Liver Lipid: The same procedure as described above was used for total liver lipid determination and estimation of radioactivity in the liver lipid.

Adipose Tissue Lipid Extraction:

Adipose tissue was extracted by grinding it in an isopropanol-heptane mixture. The extract was washed as recommended (345).

Procedure

5 g adipose was homogenized with 5 volume of the neutral extraction mixture (2% water-no acid, 78% isopropanol and 20% heptane). The mixture was allowed to settle and the upper phase was separated.

The lower phase was washed five times with blank upper phase to remove neutral fat. The washings were combined with the sample upper phase and the total volume was recorded. An aliquot or the total volume was then dried in a tared vessel under nitrogen at 40°, and the residue was dried, over phosphoric anhydride in a vacuum dessicator to constant weight. The amount of lipid was then determined gravimetrically.

An aliquot of the extract or the weighed amount of dried lipid was then combusted in the oxidizer and counted by LSC for the determination of radioactivity.

Plasma Free Fatty Acids Estimation:

Extraction of plasma long chain non-esterified fatty acids by the single extraction method of Dole et al. (345) was made by adding plasma to 5 volumes of the extraction mixture: 2% water (1 N H_2SO_4 - 0.1 volume), 78% isopropanol (4 volume), 20% heptane (1 volume).

The pH ≈ 2.5 of the lower phase thus obtained would facilitate a maximal amount of fatty acid distribution to the solvent (upper phase) and eliminate triglycerides, cholesterol and cholesterol esters from the upper phase.

Twenty ml of the extraction mixture was added to 4 ml of fresh plasma. This was shaken, allowed to stand at room temperature for ≈ 5 minutes, until two phases are clearly separated. The upper phase was siphoned off carefully without disturbing the lower phase, and was dried over nitrogen at 40° on a water bath. Radioactivity was determined either by combusting the weighed dried material or LSC of a measured aliquot of the solvent obtained by siphoning. The amount of fatty acid per 100 ml plasma was measured gravimetrically and expressed as g per 100 ml plasma.

Liver Glycogen and Glycoprotein Isolation

Liver glycogen was determined gravimetrically using the method of Bloom et al. (331) described elsewhere. An aliquot of glycogen was dissolved in 0.5 ml water and then combusted and subsequently counted in the LSC for the determination of radioactivity.

Liver glycoprotein was determined in defated liver tissue by precipitating glycogen by TCA (as per Bloom et al.) and recovering the TCA precipitate after separation of the supernatant containing glycogen. The TCA precipitate was then estimated and expressed as g% of tissue. This was used for the determination of nitrogen constant (using the Coleman Nitrogen Analyzer) which was subsequently converted into protein (i.e. glycoprotein by multiplication by 6.25)

Isolation of Plasma Protein and Non-Protein Fractions

Plasma was precipitated with 2 volumes of 10% TCA. The TCA precipitate (proteins) and the supernatant (non-proteins) were separated and the nitrogen content in each case was determined by using Coleman nitrogen analyzer (Coleman Instrument Corp., Haywood, U.S.A.). The N content was converted to the corresponding protein content by multiplying by 6.25. Thus, the protein and non-protein content in the plasma were obtained.

For the determination of radioactivity, an aliquot of the TCA precipitate and the TCA supernatant, respectively were combusted and counted, subsequently by LSC using the method described elsewhere (347).

Radioactivity measurements were made by liquid scintillation counting in an appropriate fluor, after the tissues had been combusted to $^{14}CO_2$.

10. Determination of Plasma Amino Acids

Plasma free amino acids in uremic, sham operated and restricted diet control rats were qualitatively and quantitatively determined using JEOL JLC-5AH amino acid analyser (with integrater) (Model JLC-5AH, Jeol Co., U.S.A. Incorporated, Cranford, N.J., U.S.A.). Analyses were performed under the supervision of Dr. L.P. Milligan, Department of Animal Sciences, University of Alberta.

Basic, acidic and neutral physiological standards of amino acids (Pierce Chemicals, Rockford, Illinois, U.S.A.) were used in these analysis. Each sample analyzed was the pooled plasma of eight rats. Blood samples were taken every two weeks.

The internal standards (IS), amino guanidino propionic acid (AGPA) for the basic column (short column) and norleucine for the acidic and neutral column (long column) (both resin columns), were used. A stock of 0.4 µmole/ml of IS with a final concentration (after mixing with plasma) of 0.1 µmole/ml was used in all samples. Plasma samples were prepared using the method of Thomas _et al_. (348). Solid sulfosalicyclic acid (SSA) (30 mg per ml plasma) was used for deproteinization. Combistix$^{T.M.}$ was used to test for completeness of protein removal. Internal standard was added to the protein free plasma, and the pH was adjusted to 2.5, if necessary, using lithium hydroxide (Fischer Scientific Co., Pittsburgh, Pa.).

The quantitative results were obtained by use of the internal integrater and were expressed in micromoles/ 100 ml plasma (as shown in Appendix E, page 337). Qualitative analyses were obtained by comparisons to separations of known mixtures (see Appendix E). A typical amino acid chromatogram of the uremic plasma is presented in Fig. 31.

CHAPTER IV

RESULTS

To delineate the effect of sub-total nephrectomy alone from the effect of starvation (the latter being a secondary effect of uremia), the results have been expressed in terms of the effect of sub-total nephrectomy and the effect of diet restriction. The results are presented in the following categories to facilitate discussion.

A. The Effect on BUN, SGOT, Serum CPK and Alkaline Phosphatase Activities, Hematocrit and Plasma Glucose

1. BUN

The effect of sub-total nephrectomy on the BUN has been reported in Table I. The results are grouped under two subclasses, one in which the nephrectomized rats lived throughout the 19 week period, and the other in which many animals expired before the study was complete; the second group also includes those animals in the first group. The BUN in sham operated rats (SO) also have been reported. The BUN ranges from 54±3 to 79±6 mg% in survivors, and from 62±69 mg% to 124±60 mg% in all experimental animals, for the 3 to 19 week post-nephrectomy period.

Table II reports the effect of restricted diet on BUN, as compared to SO rats. The ranges were 13±2 to

32±20 mg% in RD and 15±1 to 23±8 mg% in SO, during the 3 to 17 week period.

2. SGOT

The effect of sub-total nephrectomy and restricted diet on SGOT activity has been shown in Table III, vis-a-vis SO values.

The normal values range from 16±2 to 26±8 IU. In nephrectomized and restricted diet rats the values ranged from 7±1 to 11±2 IU, and from 10±1 to 15±5 IU, respectively.

3. Serum CPK

Serum CPK activity is reported in Table IV, showing the effects of uremia (U), and restricted diet (RD) vis-a-vis sham operated (SO) values. The normal range of serum CPK activity in SO rats is from 90±73 to 312±192 IU, while the activities in uremic and restricted diet rats range from 28±22 to 109±65 IU and from 106±26 to 171±148 IU, respectively.

4. Plasma Alkaline Phosphatase Activity

Table V reports the activities of plasma alkaline phosphatase in U, RD and SO rats. The levels in SO rats runs from 11±1 to 22±7 IU. The levels in uremic and restricted diet rats range from 11±3 to 21±4 IU and 10±2 to 19±1 IU, respectively.

5. Hematocrit

The hematocrit ratio in uremic, restricted diet and sham operated rats has been presented in Table VI. Normally in SO rats, the hematocrit ranges from 0.59±0.01 to 0.66±0.03 with consistency. In contrast, both nephrectomized and restricted diet rats showed randomized fluctuation ranging widely from 0.44±0.04 to 0.61±0.04 and 0.56±0.01 to 0.71±0.03, respectively.

6. Plasma Glucose

Table VII presents the results of plasma glucose levels in nephrectomized rats and in restricted diet rats. In all cases, 4 to 6 hour post absorptive plasma values are reported. The values in SO rats run from 66±4 to 113±23 mg%. In contrast, the ranges shown by nephrectomized and restricted diet rats are from 45±16 to 81±10 mg% and 41±15 to 93±19 mg%, respectively.

B. *In Vivo* Oxidation of ^{14}C-Glucose

In vivo oxidation of ^{14}C-glucose by nephrectomized, and sham operated rats is presented in Table VIII. The conversion of both uniformly labelled and C-1-^{14}C-labelled glucose by both of these two species is also reported. The time after injection, for the maximum rate (T_M in min.) of conversion of labelled glucose to $^{14}CO_2$, was found to be higher in the majority of the control groups than in the nephrectomized group. The total conversion of ^{14}C-glucose to $^{14}CO_2$ up to T_M in SO and nephrectomized rats was 0.5±3.3 and 2.9 to 8.1 percent of the dose, respectively. The post nephrectomy period was 3 to 6.5 weeks.

C. The Structure of Glycogen

Table IX presents the results of the structural analysis of hepatic glycogen obtained from sham operated controls as well as nephrectomized rats at the end of the 4.5 week post operative period. The average number of glucose residues per segment in nephrectomized rats was reduced to less than half (44%) the normal value where as the number of segments per mole in nephrectomized rats was increased by 68% of that of the normal. The number of non-reducing ends in nephrectomized rats increased by 67%, giving a 70% increase in the average number of branching points (i.e. 1,6-bonds) in nephrectomized rat glycogen when compared to SO values.

The EM of U, SO, and RD liver are presented in figures 27 to 29, respectively. Figure 30 shows the sites of reducing and non-reducing ends in a schematic drawing of a glycogen molecule.

D. Urinary Glucose Levels

Table X presents the data in terms of total urine volume and pH; urinary glucose levels determined both quantitatively and qualitatively; and urinary protein, qualitatively, for three consecutive 24 hour periods, 19

Fig. 27. Electron microscopy of uremic rat liver.

Fig. 28. Electron microscopy of Sham operated rat liver.

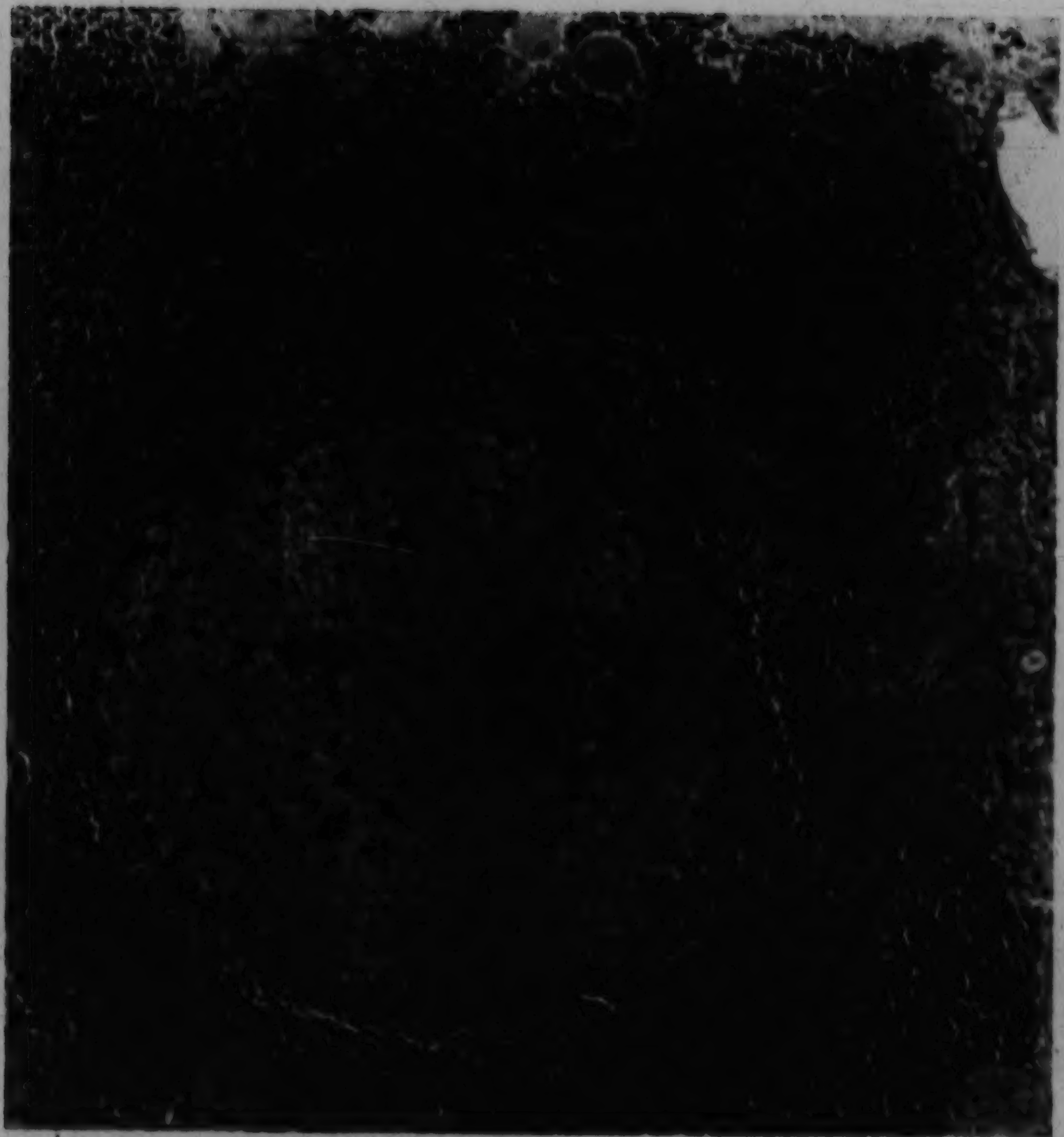


Fig. 29. Electron microscopy of restricted diet rat liver.

Fig. 30. Schematic drawing of a glycogen molecule.

Shows:

1,4 (linear and 1,6 (branching) linkages.

4 points where periodate would oxidatively cleave

points where HCOOH (formic acid) would be formed

Non-reducing and reducing terminal

n = no. of glucose units between branching.

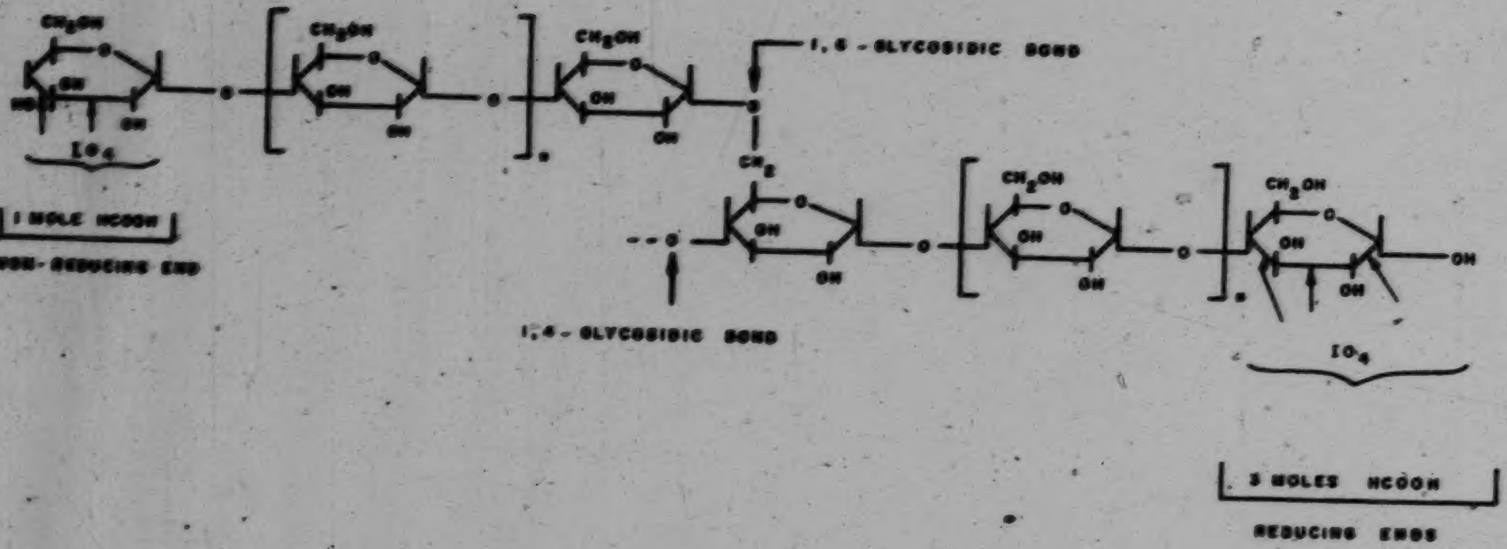
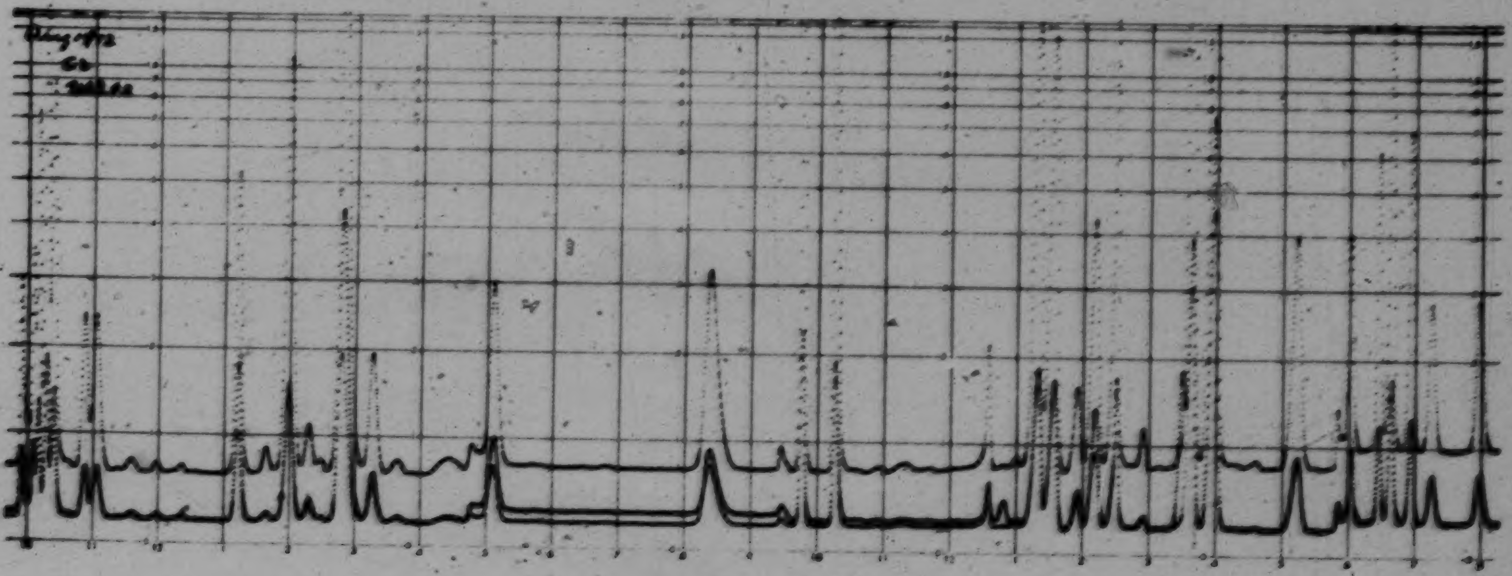

CH2OH
OH
IO4
1 MOLE HCOOH
NON-REDUCING END
n
1,6 - GLYCOSIDIC BOND
CH2
1,4 - GLYCOSIDIC BOND
IO4
3 MOLES HCOOH
REDUCING ENDS

Fig. 31. Amino acid chromatographic profile of uremic plasma.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weeks post operatively in U, RD, and SO rats. The uremic rat urine level of glucose ranged from 3±1 to 24±3 mg%. In contrast, the corresponding range of urinary glucose concentrations in RD and SO were 15±1 to 141±25 mg% and 26±2 to 103±17 mg%, respectively. The increase in the total urine volume was marked in uremic rats, although all species showed the same pH, 9.0.

E. Effect of Physical Forms of Food on Parameters

Table XI presents the effect of powder and pellet forms of diet on activities of SGOT and alkaline phosphatase, and on plasma glucose and BUN levels in uremic and sham operated rats.

The uremic SGOT ranged from 5±2 to 8±2 IU upon powder diet feeding. This is comparable to the SGOT range of 4±1 to 8±2 IU upon pellet feeding. The corresponding SGOT ranges for powder feeding and pellet feeding in SO control rats were 13±2 to 20±3 IU and 12±2 to 20±2 IU, respectively.

The alkaline phosphatase range for uremic rats on a powder diet was 7±3 to 15±4 IU; for feeding on pellets it was 7±3 to 9±2 IU. In contrast, the SO control alkaline phosphatase range for powder diet was 13±4 to 16±3 IU, and for pellets, 9±3 to 15±3 IU. Plasma glucose levels for powder and pellet diet in uremia were 72±18 to 97±13 IU and 70±4 to 95±9 IU, respectively. In contrast, the control plasma glucose levels for powder and pellets were 93±7 to 111±7 IU and 93±6 to 114±7 IU, respectively.

BUN values for powder and pellet fed uremic rats were 62±22 to 78±24 mg% and 63±20 to 115±82 mg%, respectively.

F. The Effect of Uremia and Restricted Diet on Liver Glycogen

Table XII reports the uremic liver glycogen content and the corresponding values for BUN, SGOT, serum CPK, plasma alkaline phosphatase and glucose over post operative periods of 5,9, 15, 19 and 1 to 19 weeks. The range for BUN in uremic rats ran from 68±18 to 141±30 mg%; the glycogen ranged from 5.3±3.1 to 5.9±0.2 g%. The corresponding control values for BUN were 16±2 to 22±6 mg% and for glycogen were 2.7±0.6 to 5.1±1.7 g%. The ranges for SGOT, serum CPK, plasma alkaline phosphatase and glucose level in uremia were 5±2 to 8±1 IU, 9±1 to 11±1 IU, 6±1 to 14±3 IU, and 76±12 to 90±23 mg%, respectively. The corresponding control values for SGOT, serum CPK, plasma alkaline phosphatase and glucose level were 17±2 to 23±3 IU, 20±2 to 56±16 IU, 16±1 to 21±5 IU, and 104±4 to 113±12 mg%, respectively.

Table XIII presents the liver glycogen content in restricted diet rats, having 4.5±2.6 g% for 1 to 9 weeks of the post operative period and 2.4±0.5 g% for the 10 to 19 week post operative period. The corresponding values for control (SO) were 3.5±0.7 and 2.8±0.5%, respectively.

G. The Effect of Sub-Total Nephrectomy and Restricted Diet on Hepatic Glycogen Autolysis at 25°C

Table XIV shows the percent autolysis of glycogen in the liver of U, RD and SO rats. Autolysis for a period of 30 minutes had no appreciable effect on uremic liver glycogen, whereas restricted diet and sham operated rats liver glycogens were appreciably affected (31.3±9.2% and 40.8±1.5% to 41.0±14.7%, respectively).

Autolysis for a period of 90 minutes caused a 36 percent loss of uremic liver glycogen; corresponding percentage losses of liner glycogen for restricted diet and sham operated rats were 41 and 58, respectively.

H. The Effect of Sub-Total Nephrectomy and Restricted Diet on Amylo-1,6-Glucosidase in Muscle and Liver

Table XV presents the effect of uremia and restricted diet on the muscle enzyme, Amylo-1,6-glucosidase. The results are grouped into two, one over a post operative period of 1 to 9 weeks and the other over 10 to 19 weeks. Restricted diet rats were also maintained over these periods. In uremia, the first period the activity of the enzyme remained constant (Δ+3% $P \leq .495$) while the activity for the second period decreased by 41% ($P \leq 0.05$) when compared to sham operated rats. In RD rats compared to sham operated rats, the activity increased marginally

(Δ+29%; $P \leq 0.15$) in the first period and decreased in (Δ-17%; $P \leq 0.30$) in the second period.

Table XVI depicts the results of the activity of Amylo-1,6-glucosidase obtained from the liver of U, RD, and SO rats. In the first period the activity in uremia increased marginally (Δ+17%; $P \leq 0.25$) while in the second period it decreased by 38% ($P \leq 0.10$) when compared with that in SO rat liver. Compared to the activity of the SO rat liver, the activity in RD liver decreased marginally (Δ-19%; $P \leq 0.20$) in the first period and increased marginally (Δ+17%; $P \leq 0.35$) in the second period.

I. The Effect of Uremia, and Restricted Diet on Rat Muscle and Liver Glycogen Synthetase 'I' and 'D' Enzyme Activities

Table XVII presents the activities of the enzyme glycogen synthetase 'I' and 'D' in muscle of U, RD, and SO rats. In the first 9 weeks after sub-total nephrectomy, the activity of enzyme 'I' increased by 170% ($P \leq 0.01$) whereas in the second period of up to 19 weeks it was virtually unchanged (Δ+14%; $P \leq 0.25$) when compared to the SO rats. Interestingly, glycogen synthetase 'D' in uremia in the first 9 weeks decreased by 11% ($P \leq 0.0005$) followed by a decrease of 1% ($P \leq 0.10$) in the next period when compared to the SO control. In contrast to the SO group, glycogen synthetase 'I' in the muscle of RD rats increased slightly (Δ+21%; $P \leq 0.15$) in the first 9 weeks followed by a significant decrease of 20% ($P \leq 0.05$) in the next 10 weeks; the activity of glycogen synthetase 'D' increment in the first 9 weeks was 25% ($P \leq 0.15$) followed by an increase of 47% ($P \leq 0.025$) in the next 10 weeks.

Table XVIII present the data on liver glycogen synthetase 'I' and 'D' in sub-total nephrectomized, restricted diet and sham operated rats.

The synthetase 'I' activity in uremic liver decreased by 25% ($P \leq .05$) in the first 9 weeks followed by a decrease of only 6% ($P \leq 0.0005$) in the last 10 weeks, whereas synthetase 'D' activity decreased by 66% ($P \leq 0.005$) in the first 9 weeks and decreased marginally (Δ+18%; $P \leq 0.15$) in the latter period when compared to those activities in the liver of SO rats.

In comparison with the SO liver enzyme, synthetase 'I' activity in restricted diet rat liver decreased by 22% ($P \leq 0.10$) in the first 9 weeks followed by a decrease of 19% ($P \leq 0.10$) in the last 10 weeks whereas synthetase 'D' activity decreased by 35% ($P \leq 0.10$) in the first 9 weeks and decreased only slightly (Δ-17%; $P \leq 0.15$) in the last 19 weeks.

J. The Effect of Sub-Total Nephrectomy and Restricted Diet on the α-Glucan Phosphorylase Activity of Rat Muscle and Liver

Table XIX presents the results on muscle α-glucan phosphorylase 'a' and 'b' activities in muscle of U, RD, and

SO rats. The ratio of the activity of phosphorylase 'a' to that of 'a plus b' in the muscle of uremic rat decreased by 41% in the first 9 weeks whereas in the latter period the decrease was only 12% when compared to activities in muscle of SO rats. In contrast to SO controls, the ratio of total phosphorylase activity ('a' to that of 'a plus b') in muscle of restricted diet rats decreased in the first 9 weeks by 60% whereas the decrease in the last 10 weeks was 40%.

Table XX presents the activities of liver α-glucan phosphorylase in U, RD and SO rats. In contrast to SO rat values the ratio of the activity of phosphorylase 'a' to those of 'a plus b' in uremia in first 9 weeks increased by 98% whereas in the last 10 weeks the increase is only 1.5%. The 'a' to 'a plus b' ratio in the liver of RD rats in the first 9 weeks increased by about 40% while in the second period it increased only by about 10% when compared to that ratio in the liver of SO rats.

K. The Effect of Uremia and Restricted Diet on Liver Glucose-6-Phosphate Dehydrogenase Activity

Table XXI presents the data on the activity of G-6-PDH in the liver of nephrectomized, restricted diet and sham operated rats. In the first 9 weeks the activity in the liver of uremic rats increased by 65% ($P \leq 0.005$), which was maintained in the second period (Δ+63%; $P \leq 0.0005$), when compared to the activities in SO rats liver. In contrast, the activity in the liver of RD rats was unchanged (Δ+0.7%; P≤0.49) in the first 9 weeks followed by a marginal increase of 18% (P≤0.2) in the latter period, when compared to SO rats.

L. ^{14}C-Glucose Conversion Experiment:
The Distribution of Tissue Components

Table XXII presents data on the distribution of tissue components in U, RD, and SO rats used in ^{14}C-U-D-glucose experiment. The mean weight of the residual renal mass in the U rats (2/3 of the single remaining kidney) was (2.24±0.40 g), almost equal in weight to that of a single kidney in RD (2.68±0.15 g) and SO (2.78±0.11 g) rats. The mean total liver mass of the U rat was 25% more with respect to that of RD and SO. Distribution of liver lipid in g% was highest in SO rats (2.63±0.06) followed by U (1.48±0.03) and RD (1.35%±0.05), whereas muscle lipid was highest in RD animals (4.80±0.20), followed by U (3.60±0.36) and SO (1.73±0.06%). However, the distribution of adipose tissue lipid in g% was highest in SO rats (72.3±2.52%) followed by RD (65.00±2.00%) and U (28.3±2.08%). Liver glycogen distribution was highest in uremic (8.52±0.46) followed by RD (6.51±0.34) and SO (3.67±0.18), and liver glycoprotein was highest in RD rats (52.3±0.2) followed by U (22.6±0.3) and SO (17.9±0.2). Plasma protein content per 100 ml plasma was highest in SO animals (2.84±0.66) followed by U (1.57±0.14) and RD (1.29±0.26), where as plasma non-protein nitrogen per 100 ml was highest in RD (4.30±0.10) followed by SO (3.99±0.11) and U (3.35±0.46). Plasma free fatty acid per 100 ml was highest in U rats (0.75±0.10) followed by SO (0.35±0.03) and RD (0.27±0.03).

Table XXIII reports the data on the conversion of ^{14}C-U-D-glucose into lipid, protein, glycogen and free fatty acids in different tissues of subtotally nephrectomized, sham operated and restricted diet rats 12 hours after ip injection of a dose of 10 uCi. The distribution of radioactivity per g liverlipid was highest in the RD rats (13.47±0.58 x 10^4 dpm) followed by U (9.34±0.48 x 10^4 dpm) and SO (6.14±0.38 x 10^4 dpm). Radioactivity levels per g adipose lipid and per g muscle lipid respectively followed the same pattern as that of liver lipid; that is, the highest incorporation was in RD animals (2.68±0.03 x 10^4 dpm and 6.09±0.03 x 10^4 dpm) followed by U (2.58±0.03 x 10^4 dpm and 4.30±0.60 x 10^4 dpm) and SO (0.39±0.02 x 10^4 dpm and 2.34±0.01 x 10^4 dpm), respectively.

Distribution of the ^{14}C-glucose label per g of liver glycogen was highest in RD rats (19.67±0.52 x 10^4 dpm) followed by SO (14.73±1.09 x 10^4 dpm) and U (13.30± 0.34 x 10^4 dpm). However, when the levels are expressed per 100 g of liver then the appearance of ^{14}C-glucose label in liver glycogen was highest in RD animals (being 128.25 x 10^4 dpm/100 g; 6.51 g glycogen), followed by U (113.32 x 10^4 dpm/100 g; 8.52 g glycogen) and SO (53.95 x 10^4 dpm/

100 g; 3.67 g glycogen). The EM frequently correlates with these results with respect to hepatic glycogen content of these three experimental groups. The amount of radioactivity in 100 g of liver TCA precipitate was highest in RD rats (24.25±0.76 x 10^5 dpm) followed by U (12.62±0.47 x 10^5 dpm) and SO (9.86±0.34 x 10^5 dpm). Plasma free fatty acids (per 100 ml plasma) showed the highest radioactivity in RD animals (5.21±0.51 x 10^4 dpm) followed by SO (4.37± 0.56 x 10^4 dpm) and U (1.26±0.14 x 10^4 dpm).

M. The Distribution of Radioactive ^{14}C-Glucose, as the Percent of the Dose, in Different Organs in Sub-Totally Nephrectomized, Restricted Diet and Sham Operated Rats

Table XXIV presents data of the distribution of radioactivity from ^{14}C-glucose to different organs of sub-totally nephrectomized, sham operated and restricted diet rats, as a percent of the dose. In the kidney, on a per g basis, uremic kidney has the highest activity (0.16±0.002) followed by RD (0.09±0.002) and SO (0.04±0.001). In the liver, on a per 100 g basis, the liver of RD rats shows the highest radioactivity (17.50±0.53), followed by U rats (11.52±0.59) and SO (7.68±0.25). In muscle, the order of distribution of the ^{14}C-glucose dose as a % per g of muscle was highest in RD animals (0.27±0.001) closely followed by U (0.26±0.002) and then by SO (0.10± 0.006). Per g of adipose tissue, the highest activity was in RD rats (0.08±0.001) followed by U (0.03±0.002) and SO (0.01±0.002). Uremic plasma, on a 100 ml basis contained the highest activity levels (7.19±0.10), closely followed by RD (6.29±0.08) and SO (5.02±0.08), as a % of the dose.

Per 100 ml plasma free fatty acid, the highest activity was shown in RD rats (3.29±0.33) and the lowest in U (0.79±0.09), SO having 2.93±0.54 percent of the dose.

N. The Plasma Free Amino Acids Profile

Table XXV presents the data on plasma free amino acids of uremic rats vis-a-vis sham operated rats. The results are grouped into two periods: 1 to 6 weeks and 10 to 24 weeks post nephrectomy.

The phosphoserine component showed an increment of 65% (P≤0.025) during the first period and 266% (P≤0.005) by the second period, compared respectively to the two groups of SO rats. Two derivatives of histidine, 1-methylhistidine and 3-methylhistidine are also noteworthy. 1-methylhistidine increased by 56% (P≤0.15) in the first six week period and by 147% (P≤0.005) in the second period in uremic plasma compared to SO. However, 3-methylhistidine was only found in uremic plasma and was totally absent from SO and RD plasma (Table XXVI); uremic levels were 0.83± 0.34 micromoles/100 ml plasma in the first six weeks and they increased to 1.11±0.41 micromoles/100 ml in the last 10-24 weeks.

Total essential amino acids (arginine, histidine, isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) decreased by 7 to 22 percent in the first six weeks post nephrectomy and decreased by up to 24 percent in 10 to 24 week period. Isoleucine showed negligible increases of 13 ($P\leq0.40$) and 1 ($P\leq0.475$) percent, respectively, and methionine at first decreased by 9% ($P\leq0.10$) only to increase by 12% ($P\leq0.10$) in the second period. The total essential amino acid concentration was 219 micromoles per 100 ml plasma in SO rats, and 196 micromoles per 100 ml plasma in the uremic plasma in the first period, whereas the corresponding vaues for SO and U rats in the second period were 212 and 196 micromoles per 100 ml plasma. The non-essential amino acids (alanine plus citrulline, aspartic acid, cystine, glutamic acid, glycine, hydroxyproline, proline, and tyrosine) all increased by 13 to 55 percent in uremia in the first period, and increased by 6 to 52 percent in the second period. Serine decreased by 15% ($P\leq0.20$) and 18% ($P\leq0.0125$) during the first and the second periods, respectively, when compared to SO. The total plasma concentrations of all of these non-essential amino acids were 218 micromoles per 100 ml for SO rats and 245 micromoles per 100 ml for U rats in the first period, and 229 and 261 micromoles per 100 ml for U and SO rats, respectively in the second period.

The concentration of glycogenic amino acids (alanine plus citrulline, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, hydroxyproline, methionine, proline, serine, threonine, tryptophan and valine) was to 347 micromoles/100 ml plasma in U rats and 328 micromoles/100 ml plasma in SO rats in the first period; corresponding figures for U and SO rats in the second periods were 359 micromoles/100 ml plasma and 341 micromoles per 100 ml plasma, respectively. The total concentration of glycogenic and ketogenic amino acids (isoleucine, lysine, phenylalanine and tyrosine) amounted to 82 and 86 micromoles/100 ml plasma for U and SO rats, respectively in the first period. In the second period both SO and U rats showed identical concentrations of these amino acids (79 micromoles/100 ml).

Leucine, the ketogenic amino acid, was found in higher concentration in SO rat plasma (23 micromoles/100 ml) than in U rat plasma (21 micromoles/100 ml) in the first period; the second period levels were 22 micromoles per 100 ml for SO rats and 19 micromoles per 100 ml for U rats.

In the two periods the urea cycle amino acid ornithine increased by 8% ($P \leq 0.35$) and 9% ($P \leq 0.15$) percent, and aspartic acid increased by 36% ($P < 0.15$) and 30% ($P \leq 0.10$) while, arginine decreased by 18 to 22% ($P \leq 0.15$) and 18% ($P \leq 0.0125$) in uremia when compared to SO rats.

Table XXVI compares the plasma free amino acid profile of restricted diet rats with those of sham operated rats. Due to the paucity of samples, all data presented for both SO and RD rats are for the 18 to 24 week post nephrectomy period.

Phosphoserine increased by 12% ($P \leq 0.45$) and 1-methylhistidine increased by 10% ($P \leq 0.30$) in RD rats as compared to SO controls. However, 3-methylhistidine was not detectable in either group. The total essential amino acid content in RD rats was slightly decreased (235 μmoles per 100 ml in SO rats versus 218 μmoles per 100 ml in RD rats) while the total non-essential amino acids content in RD animals was decreased (273 μmoles/100 ml in SO rats versus 243 μmoles/100 ml in RD rats).

Plasma levels of glycogenic amino acids in the RD group decreased by about 12 percent and levels of glycogenic cum ketogenic amino acids decreased by about 11 percent, when compared to SO controls. The plasma concentration of leucine, the ketogenic amino acids decreased by about 11 percent, when compared to SO controls. The plasma concentration of leucine, the ketogenic amino acid, was decreased by 8% ($P \leq 0.10$) in the RD group compared to the SO controls. Of the plasma urea cycle amino acids, ornithine decreased by 42% ($P \leq 0.0005$), arginine decreased by 10% ($P \leq 0.40$) and aspartic acid decreased by 32% ($P \leq 0.20$) in RD rats as compared to SO rats.

TABLE I

BUN FOLLOWING SUB-TOTAL NEPHRECTOMY (mg/100 ml) (NORMAL VALUE 8-20 mg/100 ml)

	PERIODS OF CHRONIC UREMIA IN WEEKS								
Survivors	3	5	7	9	11	13	15	17	19
U16	68	68	59	53	61	51	64	77	54
U17	68	70	86	55	63	61	65	75	56
U20	61	67	76	67	62	54	63	86	51
$\bar{X}$±SD	66±4	68±2	74±14	58±8	62±1	53±5	64±1	79±6	54±3
$P \leq$	0.0125	0.0005	0.0005	0.0005	0.0005	0.0005	0.0025	0.0005	0.0005
Control									
S01	22	16	20	16	30	27	21	27	21
S02	24	14	13	17	20	26	16	26	16
S03	20	18	12	21	20	21	16	21	16
S04	21	18	18	18	19	21	22	21	22
S05	27	19	21	17	18	21	19	21	19
S06	23	18	18	19	19	23	21	23	21
S07	24	15	30	21	17	14	21	14	21
S08	22	16	20	19	16	24	20	24	20
S09	24	20	15	18	20	21	20	21	20
S010	23	13	11	21	18	16	19	16	19
S011	26	18	17	14	19	19	22	--	--
$\bar{X}$±SD	23±2	17±2	18±5	18±2	20±4	21±4	20±2	21±4	20±2

...continued

TABLE I (continued)

All Experimental Animals	PERIODS OF CHRONIC UREMIA IN WEEKS								
	3	5	7	9	11	13	15	17	19
U1	37	dead	dead	--	--	--	--	--	--
U3	37	42	dead	--	--	--	--	--	--
U6	43	52	50	65	88	112	dead	--	--
U7	57	--	70	82	111	dead	--	--	--
U8	82	--	122	dead	--	--	--	--	--
U11	63	--	152	dead	--	--	--	--	--
U12	45	62	70	76	88	89	141	199	dead
U13	49	62	51	63	74	99	198	dead	--
U15	64	64	69	55	163	dead	--	--	--
U16	68	68	59	53	61	51	64	77	54
U17	68	70	86	55	63	61	65	75	56
U18	55	62	100	dead	--	--	--	--	--
U19	54	62	63	53	54	dead	--	--	--
U20	61	67	76	67	62	54	63	86	51
U21	72	60	28	dead	--	--	--	--	--
U22	68	68	76	87	77	dead	--	--	--
U23	177	dead	--	--	--	--	--	--	--
U24	50	172	53	54	54	65	74	155	dead
U25	51	57	57	53	56	78	216	111	156
U27	58	65	72	63	70	89	133	dead	--
U28	51	52	76	49	65	77	161	41	dead
$\bar{X}\pm$SD	62±29	68±29	74±28	63±12	78±29	78±20	124±60	106±54	79±51
P$\leq$	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0025

U = Uremic

SO = Sham operated control

TABLE II

EFFECT OF RESTRICTED DIET ON BUN (MG %)

Experimental Animal	PERIODS OF UREMIA IN WEEKS							
	3	5	7	9	11	13	15	17
RD1	21	60	--	--	12	13	12	14
RD2	24	33	16	13	24	23	12	11
RD3	19	21	18	25	15	20	14	22
RD4	21	16	16	16	23	19	12	30
RD5	20	--	10	15	15	24	12	--
RD6	26	--	--	--	25	21	16	--
$\bar{X}\pm SD$	22±3	32±20	15±3	18±5	19±6	20±4	13±2	19±9
$p \leq$	0.005	0.20	0.10	0.15	0.30	0.05	0.025	0.20
SO1	15	15	20	15	19	18	15	15
SO2	20	32	16	17	21	17	18	14
SO3	23	17	22	17	22	17	16	17
SO4	29	32	18	12	21	16	13	14
SO5	17	20	17	15	17	18	15	--
SO6	22	21	15	15	24	14	--	--
$\bar{X}\pm SD$	21±5	23±8	18±3	15±2	20±2	17±2	15±2	15±1

SO = Sham operated control

RD = Restricted Diet

TABLE III

SERUM GOT (IU) IN UREMIA AND RESTRICTED DIET (NORMAL VALUE 16-19 IU)

Experimental Species	PERIODS IN WEEKS AFTER SUB-TOTAL NEPHRECTOMY																	
	3		5		7		9		11		13		15		17		19	
		%•Δ		%•Δ		%•Δ		%•Δ		%•Δ		%•Δ		%•Δ		%•Δ		%•Δ
U X±SD	7±2	-60	8±3	-68	9±3	-56	7±2	-65	8±1	-60	7±2	-63	8±3	-57	11±2	-31	10±4	-40
N	10		10		16		14		7		4		7		3		6	
P≤	0.0005		0.0005		0.0005		0.0005		0.0005		0.0005		0.0025		0.025		0.025	
SO X±SD	20±4	100	26±8	100	20±1	100	21±2	100	18±1	100	19±1	100	17±2	100	16±2	100	17±2	100
N	10		10		8		8		6		3		3		3		3	
RD X±SD	15±5	-25	14±4	-46	12±2	-40	13±6	-38	21±5	+1	15±4	-22	12±2	-30	13±1	-19	10±1	-41
N	10		10		9		8		6		3		3		3		3	
P≤	0.0125		0.0005		0.0005		0.0025		0.15		0.10		0.025		0.05		0.0025	

SO = Sham operated control
U = Uremic
RD = Restricted Diet animal
SO = 100
+ = % increase
- = % decrease
%•Δ = % change

TABLE IV

SERUM CPK (IU) IN UREMIA AND RESTRICTED DIET (NORMAL VALUE 76-115 IU)

Experimental Species	PERIODS IN WEEKS AFTER SUB-TOTAL NEPHRECTOMY																	
	3		5		7		9		11		13		15		17		19	
		%*Δ		%*Δ		%*Δ		%*Δ		%*Δ		%*Δ		%*Δ		%*Δ		%*Δ
U X±SD	81±39	-74	86±59	-66	46±24	-50	80±71	-35	103±59	-32	54±29	-50	109±65	-6	28±22	-82	31±14	-81
N	19		18		16		14		9		11		9		5		6	
P≤	0.0005		0.01		0.025		0.10		0.10		0.0025		0.45		0.0005		0.0005	
SO X±SD	312±192	100	248±242	100	90±73	100	123±51	100	151±80	100	107±6	100	116±17	100	153±24	100	165±35	100
N	11		10		7		8		6		5		3		3		3	
RD X±SD	141±32	-55	171±148	-31	142±85	+51	129±31	+5	113±26	-25	121±48	+13	142±77	+22	110±20	-27	106±26	-36
N	11		11		7		5		5		3		5		3		3	
P≤	0.005		0.20		0.15		0.45		0.20		0.01		0.30		0.05		0.05	

%*Δ = % change
SO = Sham operated control
RD = Restricted diet
SO = 100
+ = % increase
- = % decrease
U = Uremia

TABLE V

PLASMA ALKALINE PHOSPHATASE ACTIVITY (IU) IN UREMIA AND RESTRICTED DIET (NORMAL RANGE 15-22 IU)

Experimental Species	PERIOD IN WEEKS AFTER SUB-TOTAL NEPHRECTOMY																	
	3		5		7		9		11		13		15		17		19	
		%°Δ		%°Δ		%°Δ		%°Δ		%°Δ		%°Δ		%°Δ		%°Δ		%°Δ
U $\bar{X}\pm SD$	21±4	+10	15±4	-32	16±3	+14	13±3	-19	12±3	0	15±6	+36	15±5	+25	11±3	-31	12±6	-14
N	10		11		14		14		12		11		9		6		5	
P≤	0.30		0.0025		0.15		0.01		0.35		0.15		0.15		0.025		0.30	
SO $\bar{X}\pm SD$	19±5	100	22±7	100	14±2	100	16±2	100	12±3	100	11±1	100	12±2	100	16±2	100	14±2	100
N	10		10		6		7		6		4		4		3		3	
RD $\bar{X}\pm SD$	12±3	-41	14±5	-36	11±3	-25	15±7	-63	13±5	+8	19±1	+72	10±3	-17	12±1	-25	10±2	-30
N	10		10		6		7		6		4		5		3		3	
P≤	0.0005		0.005		0.01		0.45		0.40		0.0005		0.20		0.05		0.025	

%°Δ = % change
SO = Sham operated control
RD = Restricted diet
SO = 100
U = Uremic animal
+ = % increase
- = % decrease

TABLE VI

EFFECT OF UREMIA AND RESTRICTED DIET ON HEMATOCRIT (RATIO)

Experimental Species	PERIODS IN WEEKS AFTER SUB-TOTAL NEPHRECTOMY																	
	3		5		7		9		11		13		15		17		19	
		%*Δ		%*Δ		%*Δ		%*Δ		%*Δ		%*Δ		%*Δ		%*Δ		%*Δ
U X±SD	0.56 ±0.07	-16	0.56 ±0.07	- 7	0.61 ±0.04	- 2	0.57 ±0.05	- 9	0.58 ±0.08	- 5	0.49 ±0.06	-26	0.55 ±0.07	-11	0.51 ±0.06	-14	0.44 ±0.04	-26
N	25		22		13		14		15		14		9		7		5	
P.	0.0005		0.05		0.20		0.0025		0.15		0.0005		0.10		0.025		0.0005	
SO X±SD	0.65 ±0.03	100	0.60 ±0.02	100	0.62 ±0.02	100	0.63 ±0.02	100	0.61 ±0.02	100	0.66 ±0.03	100	0.62 ±0.03	100	0.59 ±0.03	100	0.59 ±0.01	100
N	14		13		9		8		10		10		4		4		3	
RD X SD	0.60 ±0.02	- 8	0.60 ±0.02	- 2	0.71 ±0.03	+15	0.63 ±0.03	+ 2	0.64 ±0.02	+ 5	0.66 ±0.08	+ 2	0.62 ±0.04	+ 2	0.61 ±0.03	+ 4	0.56 ±0.01	+ 6
N	14		13		9		8		9		9		5		4		3	
P≤	0.0005		0.48		0.005		0.35		0.005		0.45		0.35		0.05		0.05	

%*Δ = % change
SO = Sham operated control
RD = Restricted diet
SO = 100
U = Uremic animal
+ = % increase
- = % decrease

TABLE VII

EFFECT OF UREMIA AND RESTRICTED DIET ON PLASMA GLUCOSE (MG%) (NORMAL VALUE 72-92 MG/100 ML)

	PERIODS IN WEEKS AFTER SUB-TOTAL NEPHRECTOMY																	
Experimental Species	3		5		7		9		11		13		15		17		19	
		%*Δ		%*Δ		%*Δ		%*Δ		%*Δ		%*Δ		%*Δ		%*Δ		%*Δ
U X±SD	74±21	-35	63±21	-24	59±20	-32	73±24	-30	60±23	-46	54±25	-30	45±16	-32	63±17	-12	81±16	-0.9
N	10		10		10		13		7		11		9		5		6	
P≤	0.0005		0.025		0.00[illegible]		0.40		0.0005		0.10		0.025		0.25		0.48	
SO X±SD	113±23	100	83±19	100	88±10	100	104±6	100	110±9	100	77±8	100	66±4	100	72±18	100	81±10	100
N	10		10		7		7		6		3		3		3		3	
RD X±SD	83±33	-27	60±13	-28	73±23	-17	93±19	-11	91±13	-18	47±3	-40	41±15	-40	64±17	-11	63±21	-23
N	10		10		5		5		5		3		4		3		3	
P≤	0.025		0.0025		0.10		0.10		0.40		0.0025		0.025		0.30		0.15	

%*Δ = % change
SO = Sham operated control
RD = Restricted diet
SO = 100
U = Uremic animal
+ = % increased
- = % decreased

TABLE VIII

IN VIVO OXIDATION OF INTRAPERITONEALLY-INJECTED ^{14}C-GLUCOSE, BY NEPHRECTOMIZED AND SHAM OPERATED RATS

Experimental Animal	Post-Nephrectomy Period	Position of C-14	Dose (μCi)	Conversion to $^{14}CO_2$	
				Time of Maximum rate (min)	% of dose by maximum time
Control*	3*	C - 1	2	50	0.5
Uremic	3	C - 1	2	20	7.9
Uremic	4.5	C - 1	2	30	6.9
Control*	6.5*	C - 1	1	80	2.9
Uremic	6.5	C - 1	1	50	5.0
Uremic	6.5	C - 1	1	60	3.7
Control*	1.5*	U	1	170	3.3
Uremic	1.5	U	1	170	8.1
Control*	4.5*	[illegible]	1	170	3.3
Uremic	4.5	U	1	135	9.6
Uremic	5	U	1	105	9.3
Control*	13*	U	1	120	1.7
Uremic	13	U	1	120	2.9

* Post-sham surgery period; these animals were the same ages as the nephrectomized animals

TABLE IX

STRUCTURE OF HEPATIC GLYCOGEN OBTAINED FROM UREMIC AND CONTROL RATS 4.5 WEEKS AFTER CONTRALATERAL NEPHRECTOMY

Parameters	Control Sample	%*Δ	Uremic Sample	%*Δ
No. of rats used				
No. of moles of glucose residue per gm glycogen	6.45×10^{-3}	100	6.41×10^{-3}	-0.62
No. of moles of reducing end per gm glycogen	5.3×10^{-6}	100	5.7×10^{-6}	+8
Av. glucose residues per mole of glycogen	1217	100	1125	-8
No. of segments per mole	71	100	119	+68
Av. no. of glucose residues per segment	17.1	100	9.5	-44.4
No. of non-reducing ends	36	100	60	+67
Av. no. of branching points (i.e. 1,6-bonds)	35	100	59	+70
Av. M. Wt. in gm	1.97×10^{5}	100	1.82×10^{5}	-8
Electron microscopy of liver	no visible abnormalities		visible deposits (glycogen)	

%* = % change
+ = % increase
- = % decrease

TABLE X

URINARY GLUCOSE LEVELS (MG%) IN UREMIC (U), SHAM OPERATED (SO) AND

RESTRICTED DIET (RD) RATS, 19 WEEKS AFTER SUB-TOTAL NEPHRECTOMY

Experimental Species	URINE COLLECTION PERIOD															
	0 - 24 Hours						24 - 48 Hours					48 - 72 Hours				
Experiment # 1				Combisitix		Enzymatic Assay			Combisitix		Enzymatic Assay			Combisitix		Enzymatic Assay
	BUN in ml	TV*	pH	Protein Mg%	Glucose	Glucose Mg%	TV*	pH	Protein Mg%	Glucose	Glucose Mg%	TV*	pH	Protein Mg%	Glucose	Glucose Mg%
U	55	43.0	9.00	300 (+++)	-ve	12±3	36.0	9.00	100 (++)	-ve	3±0.2	16.5	9.00	300 (+++)	-ve	19±2
$P \leq (V_s SO)$						0.0025					0.0005					0.0005
RD	18	14.0	9.00	300 (+++)	-ve	19±4	13.0	9.00	100 (++)	-ve	19±2	10.0	9.00	300 (+++)	-ve	15±1
$P \leq (V_s SO)$						0.05					0.0005					0.0005
SO	18	8.5	9.00	100 (++)	-ve	26±2	9.0	9.00	100 (++)	-ve	40±1	3.5	9.00	100 (++)	-ve	70±2

(Continued)

TABLE X (Continued)

		URINE COLLECTION PERIOD														
		0 - 24 Hours					24 - 48 Hours					48 - 72 Hours				
Experiment # 2	BUN in ml	TV*	pH	Protein Mg%	Glucose	Glucose Mg%	TV*	pH	Protein Mg%	Glucose	Glucose Mg%	TV*	pH	Protein Mg%	Glucose	Glucose Mg%
U	134	66.0	9.00	300 (+++)	-ve	19±1	66.5	9.00	300 (+++)	-ve	19±3	66.5	9.00	300 (+++)	-ve	12±1
P≤(V$_s$SO)						0.0025					0.0005					0.0025
RD	19	6.0	9.00	100 (++)	-ve	53±5	3.0	9.00	100 (++)	-ve	141±25	5.0	9.00	100 (++)	-ve	58±2
P≤(V$_s$SO)						0.05					0.01					0.0125
SO	18	4.0	9.00	30 (+)	-ve	68±10	4.5	9.00	30 (+)	-ve	78±9	3.0	9.00	30 (+)	-ve	103±22
Experiment # 3																
U	137	73.5	9.00	300 (+++)	-ve	24±3	81.0	9.00	300 (+++)	-ve	23±1	75.0	9.00	300 (+++)	-ve	23±1
P≤(V$_s$SO)						0.0025					0.0005					0.0005
RD	19	3.5	9.00	300 (+++)	-ve	70±8	2.0	9.00	100 (++)	-ve	105±4	2.0	9.00	300 (+++)	-ve	109±6
P≤(V$_s$SO)						0.025					0.15					0.05
SO	18	3.5	9.00	30 (+)	-ve	103±17	3.0	9.00	30 (+)	-ve	94±12	5.0	9.00	100 (++)	-ve	99±5

* = total urine volume

TABLE XI

EFFECT OF POWDERED DIET ON SGOT, ALKALINE PHOSPHATASE, GLUCOSE AND BUN IN UREMIC AND SHAM OPERATED RATS

(FIGURES OUTSIDE PARENTHESIS INDICATE POWDERED FORM AND INSIDE PARENTHESIS INDICATE PELLET FORM OF DIET EFFECT)

Experimental Species	Parameters Measured	PERIODS IN WEEKS AFTER SUB-TOTAL NEPHRECTOMY			
		9	11	13	15
U X±SD	SGOT (IU)	6±3 (6±2)	6±1 (6±2)	8±2 (8±2)	5±2 (4±1)
N		6(6)	6(3)	5(3)	4(3)
P≤		0.0005 (0.0005)	0.0005 (0.0025)	0.0025 (0.01)	0.0005 (0.0005)
SO X±SD	SGOT (IU)	14±3 (16±3)	13±2 (12±2)	17±4 (17±4)	20±3 (20±2)
N		6(7)	5(6)	6(6)	5(6)
U X±SD	Alkaline Phosphatase (IU)	13±4 (8±3)	12±5 (9±2)	15±4 (8±3)	7±3 (8±1)
N		6(5)	6(3)	5(3)	5(3)
P≤		0.15 (0.0005)	0.0025 (0.48)	0.45 (0.05)	0.025 (0.15)

TABLE XI (Continued)

Experimental Species	Parameters Measured	PERIODS IN WEEKS AFTER SUB-TOTAL NEPHRECTOMY			
		9	11	13	15
SO X±SD	Alkaline Phosphatase (IU)	16±3 (15±3)	13±4 (9±4)	15±5 (13±4)	13±4 (12±2)
N		6(7)	5(6)	6(6)	5(3)
U X±SD	Glucose (Mg%)	91±8 (95±9)	72±18 (83±14)	97±13 (93±16)	79±9 (70±4)
N		6(6)	6(6)	5(5)	4(4)
P≤		0.25 (0.48)	0.025 (0.10)	0.025 (0.025)	0.025 (0.005)
SO X±SD	Glucose (Mg%)	94±7 (94±6)	94±7 (93±6)	111±7 (114±7)	93±7 (102±19)
N		6(6)	5(5)	6(6)	5(4)
U X±SD	BUN (Mg%)	62±22 (63±20)	72±50 (97±57)	72±22 (115±83)	78±24 (69±23)
N		6(6)	6(6)	5(6)	4(4)
P≤		0.0005 (0.0025)	0.025 (0.0025)	0.0005 (0.005)	0.0005 (0.0005)

TABLE XI (Continued)

Experimental Species	Parameters Measured	PERIODS IN WEEKS AFTER SUB-TOTAL NEPHRECTOMY			
		9	11	13	15
SO X±SD	BUN (Mg%)	15±2 (15±2)	20±2 (21±6)	17±2 (17±5)	15±2 (15±4)
N		6(6)	6(6)	6(6)	5(6)

TABLE XII

EFFECT OF UREMIA ON LIVER GLYCOGEN CONTENT AND THE CORRESPONDING BIOCHEMICAL PARAMETERS ▼

Experimental Species	PERIODS IN WEEKS AFTER SUB-TOTAL NEPHRECTOMY							
	5 (n = 5)				9 (n = 2)			
	U X±SD	SO X±SD	P	%*Δ	U X±SD	SO X±SD	P	%*Δ
BUN (Mg%)	83±28	17±2	0.0005	+400	141±30 (119.0-162.0)	22±6 (17.0-26.0)	0.025	+500
Glycogen (Mg%)	5±3	4±2	0.25	+ 24	6±2 (4.0-7.1)	5±2 (3.9-6.3)	0.40	+ 10
SGOT (IU)	6±4	20±8	0.005	- 70	8±1 (7.5-8.1)	17±2 (15.6-17.6)	0.01	- 50
CPK (IU)	10±1	35±2	0.01	- 70	9±1 (8.6-9.7)	32±10 (25.1-39.6)	0.05	- 70
Alkaline Phosphatase (IU)	14±3	21±5	0.0125	- 34	6±0.1 (5.4-5.6)	16±1 (15.1-16.3)	0.0025	- 70
Glucose (Mg%)	81±4	113±12	0.0005	- 30	76±12 (66.9-84.2)	110±6 (105.6-114.5)	0.05	- 31

▼ Plasma was used in all cases except SGOT and CPK where serum was used.

SO = Sham operated control
SO = 100
U = Uremic animal
+ = % increase
- = % decrease
%*Δ = % change

TABLE XII (Continued)

15 (n = 3)				19 (n = 2)				1 to 19 (n = 12)			
U X±SD	SO X±SD		%*Δ	U X±SD	SO X±SD		%*Δ	U X±SD	SO X±SD		%*Δ
91±13	16±1	0.0005	+500	68±18 (55.0-80.0)	21±1 (20.0-22.0)	0.05	+220	92±32	18±3	0.0005	+411
6±2	3±2	0.05	+ 70	6±0.2 (5.8-6.1)	3±0.6 (2.3-3.1)	0.01	+120	6±2	4±2	0.025	+ 41
5±2	23±3	0.0025	- 79	6±2 (4.6-6.7)	21±1 (20.5-21.4)	0.005	- 73	6±2	20±6	0.0005	- 75
15±9	56±16	0.01	- 70	11±1 (10.4 12.0)	20±2 (18.0-21.0)	0.025	- 43	11±5	37±19	0.0005	- 70
10±4	17±3	0.05	- 44	9±2 (8.0-10.5)	16±1 (15.3-17.1)	0.025	- 43	11±4	19±4	0.0005	- 42
90±23	104±4	0.15	- 13	82±5 (78.7-85.4)	112±1 (111.0-113.0)	0.01	- 27	83±12	110±9	0.0005	- 34

TABLE XIII

EFFECT OF RESTRICTED DIET ON LIVER GLYCOGEN CONTENT

Experimental Species	PERIODS IN WEEKS AFTER SUB-TOTAL NEPHRECTOMY					
	1 to 9 Weeks			10 - 19 Weeks		
	BUN (Mg%)	Glycogen (gm%)	%*Δ	BUN (Mg%)	Glycogen (gm%)	%*Δ
RD $\bar{X}$±SD	13.0 ±2.0	4.5 ±2.6	+29	15.4 ±6.0	2.4 ±0.5	-15
N	4	4		5	5	
P	0.0025	0.25		0.15	0.20	
SO	22.2 ±2.9	3.5 ±0.7	100	20.0 ±5.7	2.8 ±0.5	100
N	4	4		5	5	

%*Δ = % change
SO = Sham operated control
SO = 100
RD = Restricted Diet Animal
+ = % increase
- = % decrease

TABLE XIV

PERCENT AUTOLYSIS (AT 25°C) OF GLYCOGEN OBTAINED FROM U, RD AND SO LIVER RESPECTIVELY

Experimental Animal	AUTOLYSIS PERIOD IN MINUTES	
	30*	90¥
U	non-detectable	36.2±21.4
N	3	5
P (VS SO)		0.10
SO	40.8±1.5	57.6±17.4
N	4	5
RD	31.3±9.2	40.6±25.2
N	4	5
P (VS SO)	0.20	0.25
SO	41.0±14.7	51.2±17.8
N	4	5

* 9 Weeks post nephrectomy
¥ 10-19 weeks post nephrectomy

TABLE XV

EFFECT OF UREMIA AND RESTRICTED DIET ON MUSCLE AMYLO-1,6-GLUCOSIDASE ACTIVITY (IN HERS UNITS) VALUES IN PARENTHESIS ARE IN μ MOLE GLUCOSE PER GM TISSUE PER HOUR

Experimental	PERIODS IN WEEKS AFTER SUB-TOTAL NEPHRECTOMY			
Animal	1 to 9	*%Δ	10 to 19	*%Δ
U $\bar{X}$±SE	93.1±13.7 (416.0±47.8)	+2.7 (-27)	50.8±9.6 (217.0±48.4)	-41 (-59)
N	12 (12)		15 (15)	
P (VS-SO)	0.495 (o.10)		0.05 (0.0025)	
SO $\bar{X}$±SE	90.6±15.5 (567.4±98.0)	100 (100)	86.6±14.9 (532.0±90.9)	100 (100)
N	12 (12)		15 (15)	
RD $\bar{X}$±SE	116.7±21.9 (723.5±137.2)	+28.8 (+28)	72.1±17.9 (453.0±111.3)	-17 (-15)
N	12 (12)		15 (15)	
P (VS-SO)	0.15 (0.20)		0.30 (0.30)	

*%Δ = % change
SO = 100; + = % increase; - = % decrease

TABLE XVI

EFFECT OF UREMIA AND RESTRICTED DIET ON LIVER AMYLO-1.6-GLUCOSIDASE ACTIVITY IN HERS UNIT.

Experimental Species	PERIODS IN WEEKS AFTER SUB-TOTAL NEPHRECTOMY			
	1 to 9		10 to 19	
		%*Δ		%*Δ
U X̄±SE	103.6±15.3 (649.5±97.6)	+17 (+16)	38.3± 8.7 (231.7±52.7)	-37.8 (-31.2)
N	12 (12)		15 (15)	
P (VS SO)	0.25 (0.25)		0.10 (0.10)	
SO	88.3±12.3 (560.5±79.4)	100 (100)	61.6±13.4 (380.3±82.6)	100 (100)
N	12 (12)		15 (15)	
Rd	71.8±11.4 (453.2±67.6)	-18.7 (-19)	71.8± 19.2 (427.7±116.1)	+16.6 (+12.5)
N	12 (12)		15 (15)	
P (VS SO)	0.20 (0.20)		0.35 (0.40)	

%*Δ = % change
SO = 100
+ = % increase
- = % decrease
Values in parenthesis are in μ mole glucose per gm tissue per hour.

TABLE XVII

EFFECT OF UREMIA AND RESTRICTED DIET ON MUSCLE GLYCOGEN SYNTHETASE "I" AND "D" (IN μMOLES GLUCOSE/GM/MIN)

Experimental Species	PERIODS IN WEEKS AFTER SUB-TOTAL NEPHRECTOMY							
	1 - 9		1 - 9		10 - 19		10 - 19	
	Synthetase "I"		Synthetase "D"		Synthetase "I"		Synthetase "D"	
		%*Δ		%*Δ		%*Δ		%*Δ
U $\bar{X}$±SE	1.13 ±0.26	+170	1.99 ±0.39	-10.[illegible]	0.58 ±0.07	+14	1.81 ±0.49	-0.5
N	18		18		15		15	
P (VS SO)	0.01		0.0005		0.25		0.10	
SO $\bar{X}$±SE	0.42 ±0.04	100	2.23 ±0.46	100	0.51 ±0.04	100	1.82 ±0.27	100
N	18		18		15		15	
RD $\bar{X}$±SE	0.51 ±0.07	+21	2.79 ±0.42	+25	0.41 ±0.04	-20	2.67 ±0.30	+47
N	18		18		15		15	
P (VS SO)	0.15		0.15		0.05		0.025	

%*Δ = % change   + = % increase
SO = 100   - = % decrease

TABLE XVIII

EFFECT OF UREMIA AND RESTRICTED DIET ON LIVER GLYCOGEN SYNTHETASE "I" AND "D" ACTIVITY (IN μ MOLES GLUCOSE/GM/MIN.

Experimental Species	PERIODS IN WEEKS AFTER SUB-TOTAL NEPHRECTOMY							
	1 to 9		1 to 9		10 to 19		10 to 19	
	Synthetase "I"		Synthetase "D"		Synthetase "I"		Synthetase "D"	
		%*Δ		%*Δ		%*Δ		%*Δ
U $\bar{X}$±SE	0.54 ±0.05	-25	0.78 ±0.10	-66	0.55 ±0.03	- 6	0.92 ±0.15	-18
N	18		18		15		15	
P (VS SO)	0.05		0.005		0.0005		0.15	
SO $\bar{X}$±SE	0.72 ±0.08	100	2.30 ±0.55	100	0.58 ±0.05	100	1.12 ±0.08	100
N	18		18		15		15	
RD $\bar{X}$±SE	0.56 ±0.05	-22	1.49 ±0.19	-35	0.48 0.06	-19	1.31 ±0.13	-17
N	18		18		15		15	
P (VS SO)	0.10		0.10		0.10		0.15	

%*Δ = % change
SO = 100
+ = % increase
- = % decrease

TABLE XIX

EFFECT OF UREMIA AND RESTRICTED DIET ON MUSCLE α-GLUCAN PHOSPHORYLASE a AND b ACTIVITY (X 10^2 μ MOLES GLUCOSE/GM TISSUE/MIN)

Experimental Species	PERIODS IN WEEKS AFTER SUB-TOTAL NEPHRECTOMY 1 to 9 a	b	Phosphorylase activity Ratio without AMP* / with AMP	10 to 19 a	b	Phosphorylase activity Ratio without AMP* / with AMP
U $\bar{X}$±SE	1.14±0.30	1.14±0.30	0.49	0.70±0.10	1.14±0.21	0.39
N	12	12		15	15	
P (VS SO)	0.05	0.15		0.35	0.45	
SO $\bar{X}$±SE	0.62±0.12	0.70±0.18	0.83	0.66±0.04	1.22±0.26	0.35
N	12	12		15	15	
RD $\bar{X}$±SE	0.53±0.10	1.22±0.33	0.33	0.48±0.05	1.79±0.26	0.21
N	12	12		15	15	
P (VS SO)	0.30	0.10		0.005	0.10	

* Phosphorylase activity ratio

$$\frac{\text{Without AMP}}{\text{with AMP}} = \frac{'a'}{'a' + 'b'}$$

TABLE XX

EFFECT OF UREMIA AND RESTRICTED DIET ON LIVER α-GLUCAN PHOSPHORYLASE a AND b ACTIVITY ($X\ 10^2$ μ MOLES GLUCOSE PER GM TISSUE PER MIN)

Experimental Species	PERIODS IN WEEKS AFTER SUB-TOTAL NEPHRECTOMY					
	1 to 9		Phosphorylase Activity Ratio $\frac{\text{without AMP*}}{\text{with AMP}}$	10 to 19		Phosphorylase Activity Ratio $\frac{\text{without AMP*}}{\text{with AMP}}$
	a	b		a	b	
U $\bar{X}$±SE	0.68±0.08	0.30±0.07	0.89	0.62±0.08	0.25±0.04	0.71
N	12	12		15	15	
P (VS SO)	0.05	0.05		0.10	0.15	
SO $\bar{X}$±SE	0.92±0.10	1.59±0.69	0.45	0.77±0.04	0.32±0.04	0.70
N	12	12		15	15	
RD $\bar{X}$±SE	0.74±0.09	0.35±0.06	0.64	0.76±0.07	0.23±0.03	0.77
N	12	12		15	15	
P (VS SO)	0.10	0.05		0.45	0.05	

* Phosphorylase Activity Ratio

$$\frac{\text{Without AMP}}{\text{with AMP}} = \frac{\text{'a'}}{\text{'a'} + \text{'b'}}$$

TABLE XXI

EFFECT OF UREMIA AND RESTRICTED DIET ON G-6-PHOSPHATE DEHYDROGENASE IN LIVER (IU - PER GM TISSUE PER MIN)

	PERIODS IN WEEKS AFTER SUB-TOTAL NEPHRECTOMY			
Experimental Species	1 to 9		10 to 19	
		%*Δ		%*Δ
U $\bar{X}$±SE	6.78 ±0.80	+65	3.50 ±0.16	+63
N	12		15	
P (VS SO)	0.005		0.0005	
SO $\bar{X}$±SE	4.11 ±0.43	100	2.15 ±0.21	100
N	12		15	
RD $\bar{X}$±SE	4.14 ±0.63	+0.73	2.54 ±0.35	+18
N	12		15	
P (VS SO)	0.49		0.20	

%*Δ = % change
SO = Sham operated control
SO = 100
U = Uremic animal
RD = Restricted diet animal
+ = % increase
- = % decrease

TABLE XXII

DISTRIBUTION OF TISSUE COMPONENTS IN UREMIC, SHAM OPERATED AND RESTRICTED DIET RATS USED IN ^{14}C-GLUCOSE EXPERIMENT

Experimental Species	5 WEEKS POST-OPERATIVE									
	Body Wt. (g)	%*Δ	BUN (Mg%)	%*Δ	Kidney wt. ** (g)	%*Δ	Liver wt. (g)	%*Δ	Liver Lipid (g%)	%*Δ
U X̄±SD	380±6	-1.1	107±13	+530	2.24 ±0.40	-20	17.50 ±0.63	+23	1.48 ±0.03	-44
N	3		3		3		3		3	
P (VS SO)	0.20		0.0005		0.05		0.0025		0.025	
SO X̄±SD	384±4	100	17±2	100	2.78 ±0.11	100	14.17 ±0.57	100	2.63 ±0.06	100
N	3		3		3		3		3	
RD X̄±SD	352±7	- 9	18±2	+ 6	2.68 ±0.15	- 4	13.37 ±0.40	- 6	1.35 ±0.05	-48
N	3		3		3		3		3	
P (VS SO)	0.0025		0.20		0.20		0.10		0.025	

%*Δ = % changed
SO = 100
+ = % increase
- = % decrease
** = two kidneys in all except uremic animal
Ψ = TCA precipitable
Ψ = Plasma protein = TCA ppt
ΨΨ = Plasma no-protein = TCA supernatant ; values in parenthesis by difference and outside by experiment.

TABLE XXII (Continued)

Muscle lipid (g%)	%*Δ	Adipose tissue lipid (g%)	%*Δ	Liver glycogen (g%)	%*Δ	LiverΨ glyco-protein (g%)	%*Δ	PlasmaΨ protein per 100 ml (g%)	%*Δ	PlasmaΨΨ non-protein per 100 ml (g%)	%*Δ	Plasma free fatty acid per 100 ml (	%*Δ
3.60 ±0.36	+108	28.3 ±2.08	-61	8.52 ±0.46	+132	22.6 ±0.3	+26	1.57 ±0.14 (1.65 ±0.29)	-45	3.35 ±0.46	-17.8	0.75 ±0.10	+114
3		3		3		3		3 (3)		3		3	
0.0005		0.05		0.0005		0.0005		0.025 (0.005)		0.05		0.0025	
1.73 ±0.06	100	72.3 ±2.52	100	3.67 ±0.18	100	17.9 ±0.2	100	2.84 ± 0.67 (3.11 ±0.39)	100	3.99 ±0.11	100	0.35 ±0.03	100
3		3		3		3		3 (3)		3		3	
4.80 ±0.20	+180	65.00 ±2.00	-10	6.51 ±0.34	+77	52.3 ±0.2	+192	1.29 ±0.26 (2.45 ±0.16)	-55	4.30 ±0.10	+8	0.27 ±0.03	-23
3		3		3		3		3		3		3	
0.05		0.01		0.0005		0.0005		0.01 (0.05)		0.0125		0.01	

TABLE XXIII

CONVERSION OF ^{14}C-GLUCOSE INTO LIPID, PROTEIN, GLYCOGEN AND FREE FATTY ACIDS 12 HOURS AFTER ip INJECTION (10 μ Ci ≡ 2.22 X 10^7 dpm) OF D-GLUCOSE U-^{14}C IN DIFFERENT TISSUES OF UREMIC, SHAM OPERATED AND RESTRICTED DIET RATS

Experimental Species	X 10^4 dpm per gm muscle lipid	%*Δ	X 10^4 dpm per gm adipose lipid	%*Δ	X 10^4 dpm per gm liver lipid	%*Δ	X 10^4 dpm per gm liver glycogen	%*Δ	X 10^5 dpm in TCA ppt (glycoprotein per 100 gm liver	%*Δ	X 10^4 dpm per 100 ml FFA	%*Δ
U X̄±SD	4.30±0.60	+84	2.58±0.03	+562	9.34±0.48	+52	13.30±0.34	-10	12.62±0.47	+28	1.26±0.14	-71
N	3		3		3		3		3		3	
P (VS SO)	0.0025		0.0005		0.0005		0.05		0.0025		0.0005	
SO X̄±SD	2.34±0.01	100	0.39±0.02	100	6.14±0.38	100	14.73±1.09	100	9.86±0.34	100	4.37±0.56	100
N	3		3		3		3		3		3	
RD X̄±SD	6.09±0.03	+160	2.68±0.03	+587	13.47±0.58	+119	19.67±0.52	+33.5	24.25±0.76	+146	5.21±0.51	+20
N	3		3		3		3		3		3	
P (VS SO)	0.0005		0.0005		0.0005		0.0025		0.0005		0.0005	

%*Δ = % change
U = Uremic
SO = Sham operated, 100
RD = Restricted Diet
+ = % increase
- = % decrease

TABLE XXIV

DISTRIBUTION OF RADIOACTIVITY FROM ^{14}C-GLUCOSE IN DIFFERENT TISSUES OF UREMIC, SHAM OPERATED AND RESTRISTED DIET RATS 12 HOURS AFTER ip INJECTION OF ^{14}C-U-D-GLUCOSE

Experimental Species	Per gm kidney as % dose	%*Δ	Per 100 gm liver as % dose	%*Δ	Per gm muscle as % dose	%*Δ	Per gm adipose tissue as % dose	%*Δ	Per 100 ml plasma as % dose	%*Δ	Per 100 ml plasma FFA as % dose	%*Δ
U X̄±SD	0.16±0.002	+300	11.52±0.59	+50	0.26±0.002	+160	0.03±0.002	+200	7.19±0.10	+43	0.79±0.09	-73
N	3		3		3		3		3		3	
P (VS SO)	0.0005		0.0005		0.0005		0.0005		0.0005		0.0025	
SO X̄±SD	0.04±0.001	100	7.68±0.25	100	0.10±0.006	100	0.01±0.002	100	5.02±0.08	100	2.93±0.54	100
N	3		3		3		3		3		3	
RD X̄±SD	0.09±0.002	+ 55	17.50±0.53	+128	0.27±0.001	+170	0.08±0.001	+700	6.29±0.08	+21	3.29±0.33	+12
N	3		3		3		3		3		3	
P (VS SO)	0.0005		0.0005		0.0005		0.0005		0.0005		0.475	

%*Δ = % change
U = Uremic
SO = Sham operated, 100
RD = Restricted Diet
+ = % increase
- = % decrease

TABLE XXV

EFFECT OF UREMIA ON PLASMA FREE AMINO ACIDS (μ MOLES PER 100 ML PLASMA)

Amino Acids	PERIODS IN WEEKS AFTER SUB-TOTAL NEPHRECTOMY							
	1 to 6 (1, 3, 4, & 6)ψ				10 to 24 (10, 14, 18 & 24)			
	U (n = 4)ψ	SO (n = 3)**			U (n = 4)ψ	SO (n = 4)**		
	X±SD	X±SD	P	%*Δ	X±SD	X±SD	P	%*Δ
Phosphoserine	0.51±0.13	0.31±0.02	0.025	+65	1.32±0.49	0.36±0.12	0.005	+266
Taurine	68.10±18.90	57.50±5.80	0.20	+19	74.0 ±19.80	69.0±8.10	0.35	+7
Urea	1727.00±864.58	878.20±201.50	0.10	+97	3745.06±1151.08	870.66±6.55	0.0025	+330
Hydroxy Proline	8.19±2.68	5.73±0.20	0.10	+43	6.90±0.78	5.35±0.53	0.01	+30
Aspartic Acid	3.54±1.30	2.61±0.12	0.15	+36	3.35±0.82	2.58±0.16	0.10	+30
Threonine	34.50±8.56	41.00±6.68	0.20	-16	36.35±3.85	37.26±3.24	0.40	-2
Serine	31.39±8.70	36.87±0.84	0.20	-15	26.94±2.75	32.66±2.59	0.0125	-18
Asparagine	9.93±1.38	11.18±1.46	0.15	-11	8.92±0.89	11.06±0.98	0.01	-20
Proline	31.33±8.06	24.60±11.25	0.20	+27	33.58±2.25	31.68±4.81	0.25	+6
Glutamic Acid	27.38±20.08	17.69±0.31	0.25	+54	17.08±3.48	16.02±3.33	0.35	+6
Glycine	50.19±7.42	49.93±10.95	0.4875	+0.5	53.81±6.92	41.34±2.34	0.01	+30
Alanine plus Citrulline	89.50±21.28	68.42±26.68	0.15	+31	103.67±6.98	85.20±4.21	0.0025	+22
α-amino adipic acid	2.38±0.62	1.68±0.03	0.10	+41	2.52±0.52	1.14±0.30	0.0025	+121
α-amino-n-butyric acid	0.38±0.04	0.45±0.02	0.025	-13	0.33±0.14	0.39±0.05	0.25	-15

U = Uremia
SO = Sham operated, 100
RD = Restricted Diet

....(continued)

TABLE XXV (Continued)

Amino Acids	PERIODS IN WEEKS AFTER SUB-TOTAL NEPHRECTOMY							
	1 to 6 (1, 3, 4, & 6)▼				10 to 24 (10, 14, 18 & 24)			
	U (n = 4)▼	SO (n = 3)**			U (n = 4)ψ	SO)n = 4)**		
	X̄±SD	X̄±SD	P	%*Δ	X̄±SD	X̄±SD	P	%*Δ
Valine	24.36±4.54	27.44±1.29	0.15	-11	21.07±1.24	27.77±0.42	0.0005	-24
Cystine	2.03±0.31	1.80±0.01	0.15	+13	5.38±3.13	3.53±2.79	0.25	+52
Methionine	8.71±0.76	9.52±0.37	0.10	-9	10.11±1.21	8.97±0.63	0.10	+12
Isoleucine	12.47±2.03	11.98±0.52	0.40	+13	11.92±1.24	11.87±0.10	0.475	+1(+0.5)
Leucine	21.38±3.78	23.05±1.59	0.25	-8	18.61±1.63	21.82±1.46	0.025	-15
Tyrosine	11.09±1.64	10.35±0.53	0.25	+7	9.81±0.66	10.96±1.79	0.15	-10
Phenyl-alanine	9.02±1.32	9.95±0.23	0.15	-9	9.42±0.71	9.31±0.85	0.45	+1
Ornithine	21.43±4.35	19.91±4.81	0.35	+3	14.92±0.87	13.65±1.67	0.15	+9
Ammonia	47.36±9.59	33.36±7.69	0.10	+41	43.34±9.06	38.76±11.71	0.30	+12
Lysine	49.74±9.03	53.23±4.13	0.30	-7	47.85±2.26	46.96±4.40	0.40	+2
1-methyl-histidine	1.70±0.79	1.09±0.06	0.15	+56	3.56±0.80	1.44±0.24	0.005	+147
Histidine	7.39±1.44	7.32±0.76	0.475	+1	7.24±0.39	8.12±0.24	0.01	-11
3-methyl-histidine	0.83±0.34	absent			1.11±0.41	absent		
Anserine	8.95±2.27	10.63±0.39	0.15	-16	5.70±0.66	9.02±1.46	0.005	-37
Arginine	15.92±5.72	20.64±3.58	0.15	-22	21.06±1.83	25.72±2.08	0.0125	-18
Tryptophan	12.35±0.05	14.88±0.95	0.005	-17	12.14±0.19	14.64±1.22	0.005	-17

%*Δ = % change
▼ = Every sample represents the pooled plasma from 8 individual animals
** = Every sample represents the pooled plasma from 8 individual animals
+ = % increase
- = % decrease

TABLE XXVI

EFFECT OF RESTRICTED DIET ON PLASMA FREE AMINO ACIDS (μ MOLES PER 100 ML PLASMA)

	PERIODS IN WEEKS AFTER SUB-TOTAL NEPHRECTOMY					
	1 to 24 (18 & 24)		1 to 24 (18 & 24)			
	RD (n = 2)▼		SO (n = 2)▼			
	Range	X̄±SD	Range	X̄±SD	P	%*Δ
Phosphoserine	0.48-0.79	0.64 ±0.22	0.32-0.81	0.57±0.35	0.45	+12
Taurine	63.87-109.14	86.51 ± 32.01	110.50-110.66	110.58±0.13	0.20	-21
Urea	766.02-799.93	782.98 ± 23.98	876.00-926.36	901.18±35.61	0.05	-13
Hydroxyproline	3.04-4.19	3.62 ± 0.81	5.60-5.67	5.64±0.05	0.05	-36
Aspartic Acid	1.71-3.46	2.59 ± 1.24	3.70-3.85	3.78±0.11	0.20	-32
Threonine	30.96-35.26	33.11 ± 3.04	40.80-41.21	41.00±0.29	0.05	-19
Serine	37.21-40.03	38.62 ± 1.99	39.90-40.38	40.14±0.34	0.20	- 4
Asparagine	10.84-12.98	11.91 ± 1.51	12.85-12.92	12.89±0.05	0.25	- 7
Proline	22.02-29.97	26.00 ± 5.62	30.95-31.54	31.25±0.42	0.20	-17
Glutamic Acid	7.20-14.16	10.68 ± 4.92	16.99-17.65	17.32±0.47	0.10	-37
Glycine	48.55-52.26	50.41 ± 2.62	59.71-60.02	59.87±0.22	0.025	-16
Alanine plus Citrulline	74.31-108.34	91.33 ± 24.06	95.30-95.40	95.35±0.09	0.15	- 4
α-amino adipic acid	0.66-1.02	0.84 ± 0.26	0.93-0.95	0.49±0.01	0.35	-10
α-amino-n-butyric acid	0.45-0.62	0.54 ± 0.12	0.42-0.45	± 0.44±0.02	0.20	+23
Valine	26.44-29.81	28.13 ± 2.38	31.09-31.11	31.10±0.02	0.15	-10

....(continued)

TABLE XXVI (Continued)

	PERIODS IN WEEKS AFTER SUB-TOTAL NEPHRECTOMY					
	1 to 24 RD (n = 2)▼*			1 to 24 SO (n = 2)▼*		
	Range	X̄±SD	Range	X̄±SD	p	% Δ
Cystine	7.44-7.97	7.71±0.38	7.79-7.80	7.80±0.007	0.40	- 1
Methionine	9.19-9.25	9.22±0.04	9.68-9.71	9.70±0.02	0.005	- 5
Isoleucine	10.48-13.37	11.93±2.04	14.05-14.10	14.08±0.04	0.15	-15
Leucine	21.48-23.10	22.29±1.15	24.28-24.33	24.31±0.04	0.10	- 8
Tyrosine	10.20-13.25	11.73±2.16	12.15-12.30	12.23±0.11	0.40	- 4
Phenylalanine	7.59-7.64	7.62±0.04	10.28-10.39	10.34±0.08	0.0005	-26
Ornithine	9.31-9.81	9.56±0.35	16.36-16.47	16.42±0.08	0.0005	-42
Ammonia	38.04-89.93	63.99±36.69	34.63-34.68	34.66±0.04	0.20	+85
Lysine	47.26-56.90	52.08±6.82	56.67-56.70	56.69±0.03	0.25	- 8
1-Methyl histidine	1.32-1.84	1.58±0.37	1.42-1.43	1.43±0.007	0.30	+10
Histidine	8.54-9.61	9.08±0.76	9.05-9.12	9.09±0.05	0.49	-0.1
3-Methyl histidine	▼	▼	▼	▼		
Anserine	10.72-11.17	10.95±0.32	11.66-11.72	11.69±0.04	0.05	- 7
Arginine	15.69-27.94	21.82±8.66	24.26-24.30	24.28±0.03	0.40	-10
Tryptophan	12.68-13.38	13.03±0.50	13.92-13.98	13.95±0.04	0.10	- 7

▼* = each sample represents the pooled plasma for 8 animals. Samples were taken from animals of the same age, 18 and 24 weeks after sham surgery and diet restriction.
▼ = only found in uremic plasma and absent from both SO and RD plasma
%Δ = % change
+ = % increase
- = % decrease

CHAPTER V

DISCUSSION

In this research we were dealing with sick, surgically stressed, and partially starved animals, and consequently we were confronted with certain extraordinary problems. For example, obtaining a fasting blood sample was hindered by a high rate of mortality in fasted animals, and determination of a number of parameters in the same pathway was limited by plasma sample size factors due to the anaemic condition of the rats. Furthermore the uncertainty of the progression of uremia in sub-totally nephrectomized rats and the consequent spread of data due to individual variation presented a major obstacle to data analysis. For this reason, changes which are associated with $P \leq 0.10$ ($2P < 0.20$) have been arbitrarily considered significant for the purposes of discussion.

The BUN is an acceptable clinical index for monitoring the progressive deterioration of CRF, and it was studied in all rats. However, we found in many cases that compensation of the BUN occurred; that is, elevation of the BUN was temporary and the other uremic symptoms persisted. Therefore, we studied a number of other parameters to which a clinical chemist would have ready access: SGOT, serum CPK, PAP, hematocrit, plasma glucose, and plasma amino acids.

This research was undertaken to investigate the possibility of an alternate parameter being used as an index to monitoring the deteriorating condition of the nephrectomized rats.

In uremia disturbances of the nutritional homeostasis prevail, and are known to grossly affect the metabolism of protein and carbohydrate. In addition, malnutrition can influence clinical parameters such as SGOT. To determine the influence of decreased dietary intake on the serum, plasma, and whole blood characteristics of uremia rats, rats with restricted intake of the normal laboratory diet were used as controls in addition to sham operated controls.

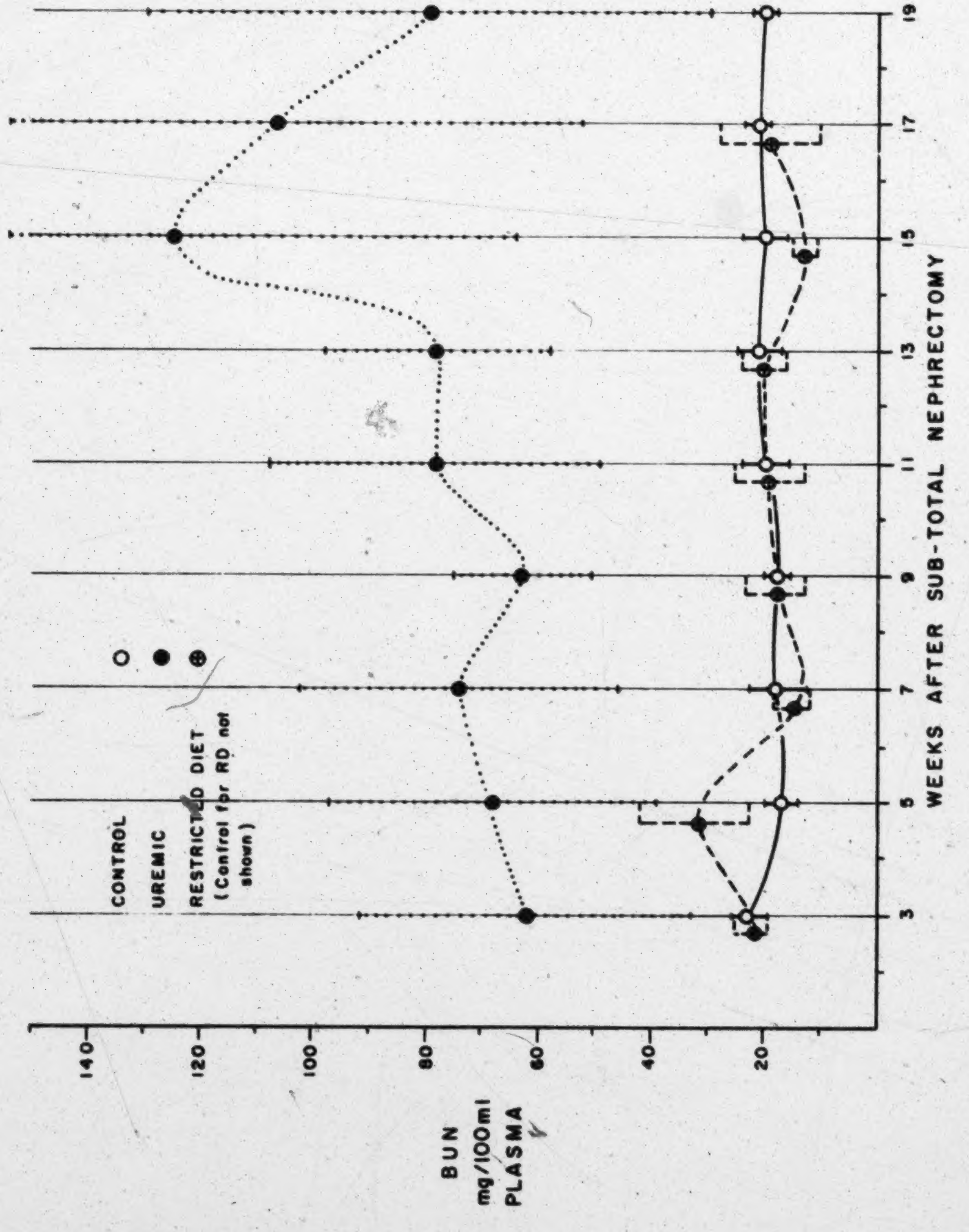
The impairment of glucose metabolism has been implicated in the uremic condition. Although this subject has been widely studied both clinically and in experimental animals, two initial findings prompted further investigation. Thus, evidence of apparent hypoglycemia in the presence of abnormal, excessive hepatic glycogen deposition led to an investigation of glucose utilization _in vivo_, and glycogen cycle enzymes _in vitro_ .

The results are discussed below with respect to each parameter measured. The significance of the results in relationship to each other is presented.

1. BUN (Table I and II)

The examination of the BUN levels (Table I) of those rats which survived all through the 19 week period shows that their BUN ranges from 52±5 to 79±6 mg%. The BUN at the end of

Fig. 32. Effect of sub-total nephrectomy and restricted diet on BUN.


BUN
mg/100ml
PLASMA
140
120
100
80
60
40
20
CONTROL ○
UREMIC ●
RESTRICTED DIET ⊕
(Control for RD not shown)
3
5
7
9
11
13
15
17
19
WEEKS AFTER SUB-TOTAL NEPHRECTOMY

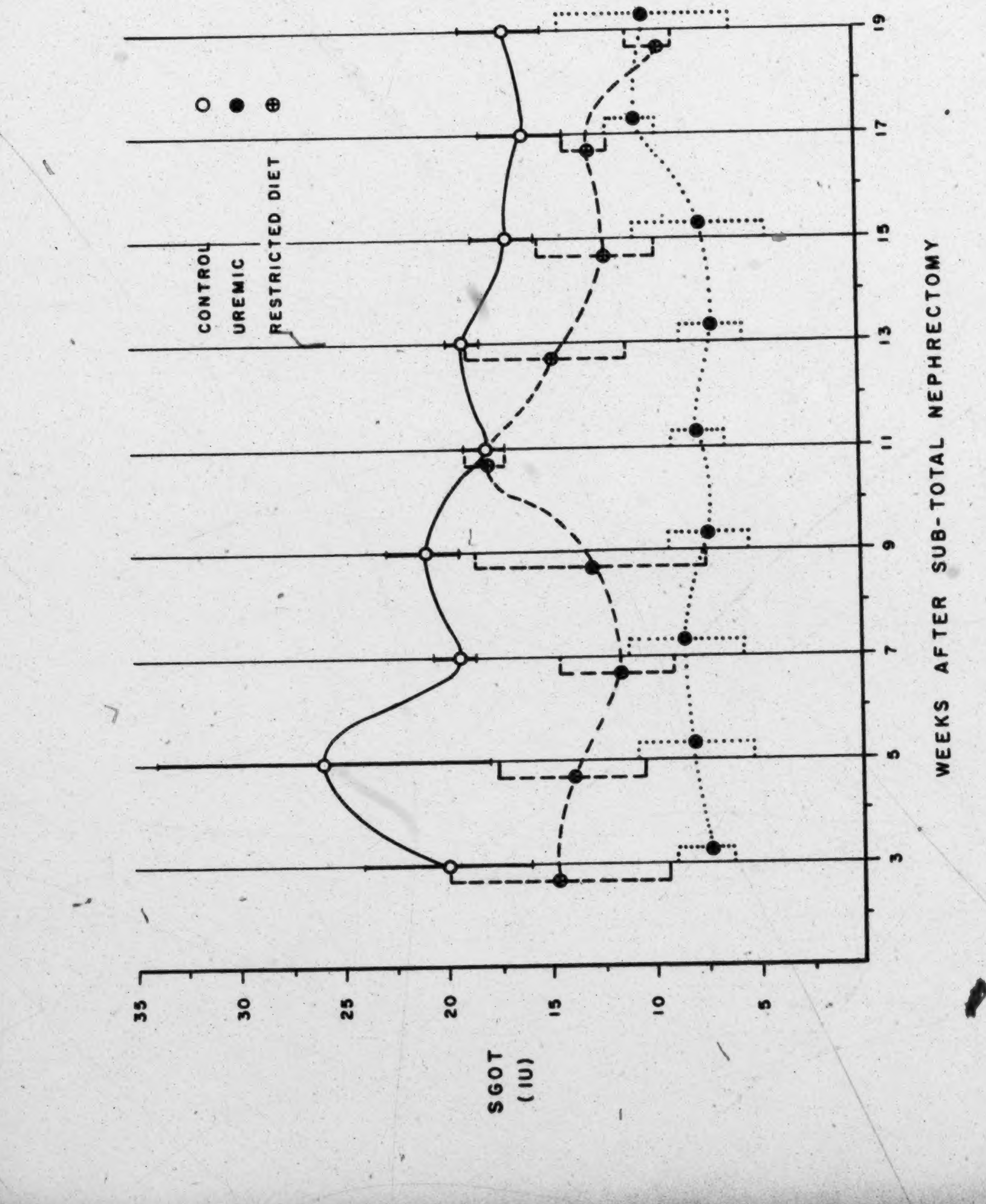
the 3rd week was 66±4 mg% and on the 19th week, 54±3 mg%. The reason for survival was probably due to hypertrophy of remaining renal mass with a decrease in BUN though the uremic symptoms still prevailed as seen by examination of other parameters. Most of the rats died as soon as their BUN rose to 100 mg%. In the table, rats marked 'dead' with a BUN higher or less than 100 mg% were not dead, but were killed for use in the experiment. The range of the BUN among these rats was 62±29 to 124±60 mg% and those which died of uremia had BUN levels between 112 and 198 mg%. For control (SO) and RD rats, the ranges of BUN are 15±1 to 23±8 and 13±2 to 32±20, respectively (Table II).

Table II shows that restricted diet does not have any significant effect on the BUN level. However, in the majority of the restricted diet rats the BUN levels show a slightly downward trend in comparison to sham operated control. This may reflect decreased activity of the urea cycle enzymes, as is evident from the analysis of the plasma amino acid profile of RD vis-a-vis SO, particularly the concentrations of aspartic acid and glutamic acid which show decreases (36% and 37%, respectively). Urea is the most important final product of protein metabolism, and its low level in restricted diet rats is expected.

2. SGOT (Table III)

SGOT levels in sub-totally nephrectomized rats have been shown to be decreased by 56 to 66% in the first 11 weeks and by 40 to 63% in the 12th to 19th weeks, when

Fig. 33. Effect of sub-total nephrectomy and restricted diet on SGOT.


SGOT
(IU)
35
30
25
20
15
10
5
3
5
7
9
11
13
15
17
19
WEEKS AFTER SUB-TOTAL NEPHRECTOMY
○ CONTROL
● UREMIC
⊕ RESTRICTED DIET

compared to SO groups. In contrast, the corresponding decrease of SGOT in RD rats was between 27 and 46% in the first 11 weeks as between 22 and 44% in the 12th to 19th week, compared to SO controls. This demonstrates that this decrease is due to the cummulative effect of both starvation and uremia. The SGOT levels in U rats were from 15 to 62% lower, during the 3rd to 17th week, than corresponding levels in RD rats. It can thus be seen that uremia per se, is largely responsible for decreases in SGOT, although the anorexia associated with uremia has a significant influence. The most interesting fact is that this decrease in the SGOT level is independent of the BUN level; that is, this decrease persists even when the BUN decreased suggesting that SGOT may have some potential for a complementary index to monitor the progress of uremia.

Transamination provides a means of redistribution of nitrogen; the two most important members of the SGOT system are asparate and oxaloacetate, which are involved through the urea cycle and the TCA cycle for the synthesis of urea.

In urea synthesis, the entry of the first N as NH_3 comes from carbamyl PO_4 and the second nitrogen is derived from the amino nitrogen of aspartic acid. Aspartic acid is formed by transamination of glutamate, and the fumaric acid formed in the arginosuccinase [which elevates in uremia (146)] reaction can be hydrated to malic acid and reoxidized to oxaloacetic acid in the citric acid cycle. The OAA can then acquire a new amino acid group by transamination, with aspartate formation and repetition of the sequence. This excessive urea formation may deplete SGOT, and may be responsible for the decreased SGOT in the U rats. This observation of a decrease of SGOT activity in uremia prompted us to determine the plasma free amino acid levels, including aspartate, in sub-totally nephrectomized and restricted diet rats. The plasma levels of SGOT in RD rats were almost double the plasma levels of SGOT in U rats. This correlates well with the observed plasma concentrations of aspartic acid and the BUN in RD and U rats (Table XXVI and XXV). These tables show that the aspartic acid concentration in RD plasma is decreased by 31% and glutamic acid (from which aspartic is formed by transamination) is decreased by 37%, whereas aspartic acid concentrations and glutamic acid concentrations are respectively increased by 30 to 36% and 55% in uremic plasma.

The elevated aspartate levels seen in the U rats are incompatible with SGOT unless the aspartate is generated via an alternate pathway, for example, via asparagine. The substrate inhibition (by high concentrations of OAA) may also cause depletion of SGOT.

Another factor which should be considered is the endocrine hormone level in plasma. Growth hormone is known to be elevated in the plasma of fasting patients with severe renal failure (282). Growth hormone injected into normal and hypophysectomized rats reduced muscle GOT (374) but injection of cortisone into rats elevated both glutamic-oxaloacetate and glutamic-pyruvic transaminases. However, when injected into the mouse, cortisone decreased liver GOT but increased kidney and heart GOT (374,375). Hydrocortisone or cortisol given to lymptocyte suspensions inhibited SGOT activity (376).

The progressive decrease of SGOT levels in uremia may therefore, be due to substrate inhibition (high levels of aspartate, glutamate, α-ketoglutarate, oxaloacetate) and/or elevation of growth hormone or disturbances in cortisol, all of which we have not explored as yet.

Kokot _et_ _al_. (350) during hemodialysis of four hours duration observed no change in SGOT. However, 0 to 96% inhibition of SGOT by aqueous solutions of 10^{-2} to 10^{-4} M of 20 to 24 special phenolic acids identified in human serum or dialysates have been reported (16).

The occurence of several glucogenic amino acids and of metabolites of the TCA cycle in aminonucleoside-induced nephrotic rats has been reported (202) in which both fed and fasted nephrotic rats showed increased concentrations of aspartate, malate, oxaloacetate, glutamate and α-ketoglutarate. These increases in the concentrations of aspartate and oxaloacetate also support our contention that a decrease in SGOT may probably be due to substrate inhibition in sub-totally nephrectomized as well as in restricted diet rats [($p \leq 0.025$ to 0.0005) and ($P \leq 0.10$ to

0.0005) respectively as compared to SO controls].

Elevation of SGOT in rat liver 48 hours after bilateral nephrectomy has also been reported (352). These studies used female rats and the determinations were made in liver only, 24 to 48 hours after bilateral nephrectomy. These rats were in an acute, terminal condition quite different from our model.

3. CPK (Table IV)

Serum CPK activity in sub-totally nephrectomized rats shows a tendency for a wide range of decreases (35 to 81%) from 3rd week to the 19th week post surgery, compared to SO rats. In contrast, the serum CPK activity in restricted diet rats shows erratic changes, with a range of 25 to 55% decrease in the 3rd, 17th, and 19th weeks, and a 13% increase in the 13th week.

It is difficult to explain this kind of fluctuation in RD rats. It may be that in the uremic there is a constant inhibition of CPK due to accumulation of creatine, whereas fluctuations in RD rats reflect the constant efforts of the animals to control the homeostasis of muscle energy.

CPK is a muscle specific enzyme and its activity reflects the energy level in the muscle. This is because the muscles possess an anaerobic mechanism for the rapid synthesis of ATP to meet the demand for energy. Resting skeletal muscle has 4 to 6 times as much phosphocreatine as

ATP. However, the typical changes in serum CPK activity level possibly indicate the fluctuating levels of adenine nucleotide (ADP and ATP), as Lohman (213) demonstrated that muscle does not possess an enzyme system for the catalysis of creatine phosphate unless adenine nucleotides are present. In view of this it may be that the depletion of CPK activity reflects the absence or inadequacy of adenine nucleotides.

It is interesting to note that both CPK and SGOT (which elevates in myocardial infarction and reflects the skeletal and cardiac muscle damage) are found to be diminished in both sub-totally nephrectomized as well as restricted diet rats. However, the increasing replacement of muscle with fatty and connective tissue completely ceases the occurrence of pathologic enzyme activities (351).

4. Alkaline Phosphatase (Table V) (Orthophosphoric Monoester Phosphohydrolase, EC 3.1.3.1)

In sub-totally nephrectomized rats the plasma alkaline phosphatase activity (PAP) shows a significant tendency of decrease (34%) in the 5th week followed by upward and downward tendency in the alternate fortnight, respectively up to the 17th week. In contrast, the PAP activity in RD rats plasma showed a significant tendency of decrease ranging from 21 to 41%, as compared to SO rats plasma activity. Kokot *et al*. (352) observed, increased PAP activity during hemodialysis of 24 hour duration as the inhibitor molecules were briefly removed. In the normal species, serum alkaline phosphatase is derived from three sources: bone, liver, and intestine. The activity increases from birth through puberty to correlate with the growth rate of bone. Elevation of the enzyme occurs in biliary obstruction (both intra and extrahepatic), viral hepatitis, portal cirrhosis, drug induced cholestasis and alcoholism. This is used diagnostically to a lesser extent in regenerative bone diseases, bone tumors, paget's disease, hyperparathyroidism rickets and osteomalacia (355). Pyrophosphates (PP_i), inhibitors of soft-tissue calcification and regulators of calcium homeostasis are known to regulate PAP and play an important role in osteodystrophy in CRF. Serum PP_i (S-PP_i) values in chronic hemodialysis did show direct correlation with the calcium phosphorus product, and inversely correlation with the serum alkaline phosphatases; for example, the S-PP_i rise is attributed to the marked decrease in alkaline phosphatase post operatively (in post renal transplant patients) (356). An increase in serum inorganic phosphate has been suggested to increase S-PP_i by inhibiting pyrophosphatases (357).

There is good evidence to suggest that calcium transfer across the intestinal and bone cells involves a calcium dependent ATPase system which may be identical with alkaline phosphatase. The latter has long been known to increase at the brush border of the intestinal cells in response to Vitamin D (358). Low 1,25-DHCC levels in uremia may be a significant contributing factor to the serum increase of PP_i (359) since pyrophosphatase activity regulates tissue and serum PP_i levels (360).

It has been suggested that the inverse correlation between S-PP_i and serum alkaline phosphatase may be especially interesting in the etiology of osteodystrophy. It is known that a rise in bone alkaline phosphatase indicates osteoblastic activity, an effort by diseased bone to heal and calcify (361). Thus, serum alkaline phosphatase is one of the best parameters other than the radiographic observation, to follow the course and severity of osteodystrophy (362). Since alkaline phosphatase acts as pyrophosphatase, higher alkaline phosphatase should accompany the lower PP_i (363). David et al. observed that the drop in PP_i during dialysis is associated with high serum alkaline phosphatase. There is also an inverse relationship between the dose of corticosteroids and S-PP_i (356). Bombar et al. (364) studied the effect of Ca infusion on changes of PAP in CRF during dialysis and observed, contrary to others, that Ca infusion lowered PAP. Most of these observations support our findings of lower PAP in sub-totally nephrectomized rats, but our findings in PAP in RD also suggest that the lower value is due to the cummulative effect of both uremia and starvation (a secondary effect of uremia).

It may be said then that alkaline phosphatase reflects the etiology of osteodystrophy; a rise indicates osteoblastic activity and a fall reflects hypocalcemia, low 1,25-DHCC levels in plasma, and high S-PP_i, with the onset of bone disease.

5. Hematocrit (Table VI)

The hematocrit in sub-totally nephrectomized rats varies from 0.44 to 0.56, in contrast to the values of sham operated rats and restricted diet rats which varied from 0.56 to 0.65 and 0.56 to 0.71, respectively. Hematocrits for U and RD rats were not significantly different from SO rat hematocrits at the 7th and 11th weeks, and the 5th, 9th, 13th and 15th weeks, respectively.

In general, the pattern of apparent hemodilution in uremics is indirect evidence of the microcitic anemia commonly associated with chronic renal disease. On the other hand, the apparent pattern of hemoconcentration in the RD is indirect evidence of the decreased circulating plasma associated with decreased plasma proteins secondary to starvation.

It would thus seem that the uremic condition and not the starvation directly caused the decreased hematocrit.

John et al. (368) correlated the residual renal function in patients receiving regular hemodialysis with the hematocrit, with a mean value of 0.22 to 0.28. They also correlated increased predialysis creatine (>18 mg%) with H_{ct} of 0.14 to 0.18. However, we observed hemoconcentration in RD with approximately the same (normal) BUN as seen in SO rats, whereas hemodilution was present in U rats with elevated BUN. Duffy et al. (185) reported that testosterone increases H_{ct} and erythropoietin in hemodialysis patients with polycystic kidney disease. In other non-polycystic kidney disease, they also found that testosterone and dialysis increased the H_{ct} but a maximum response required four months. Our results of decrease in H_{ct} in sub-totally nephrectomized are in agreement with their observation in human beings.

Erythropoietic activity, especially in combination with other signs of possible graft failure, has been suggested to be a useful indicator of transplant dysfunction (365). Gral et al. observed that the early appearance of erythropoietic activity, erythropoietin (EP), following cadaveric renal homotransplantation seems to be indicative of a reliable graft. Erythropoietin usually fell gradually within several weeks as the hematocrit increased and creatinine decreased. They also suggested that any sudden early rise or drop in EP as followed by a drop or rise in H_{ct} after transplantation might indicate acute graft failure: bleeding, obstruction, or rejection (366). Some degree of folate deficiency, accompanied by a small reticulocytosis has been implicated with a rise in hematocrit (367). Since H_{ct} screens blood dyscrasias, and computes mean corpuscular volumes and the mean corpuscular hemoglobin concentration, the decreasing tendency in uremia may measure loss of hemoglobin by systemic bleeding. Of course, by H_{ct} measurement, evaluation of anemia and polycythemia can be made without red cell counts. However, hemodilution may be due to physiologic hydremia, where a falling H_{ct} may not necessarily indicate anemia in the sense of a reduction in total number of circulating red cells. Furthermore, hemoconcentration may be due to shock, or to raised H_{ct} even though a considerable proportion of total red cell mass has been lost through hemorrhage.

6. Plasma Glucose (Table VII)

Our findings of plasma glucose levels in the sub-totally nephrectomized rats show a mean value of 45±16 to 74±21 mg%, over a period of 3 to 18 weeks post nephrectomy. The mean value of plasma glucose by the same method in SO rats is 66±4 to 113±23 mg% (Table VII). The U rats were thus significantly hypoglycemic except at the 9th and 17th week periods. In contrast, the mean values of plasma glucose in restricted diet rats over the same period of 3 to 19 weeks ranged from 41±15 to 93±19 mg%; these rats were significantly different from SO rats only at weeks 11, 17 and 19. Both sub-totally nephrectomized rats and restricted diet rats show a tendency toward hypoglycemia. The plasma sample was collected in the 4 to 6 hour post-absorptive state, as high mortality among the nephrectomized rats prevented us from taking fasting plasma samples. Our results, showing hypoglycemia in uremic rats, reflects the utility of a true glucose-specific hexokinase method. This is in agreement with the recent findings of Weisinger et al. (369), who define glucose tolerance in subjects with chronic renal failure using glucose-specific methodology. They showed that fasting glucose concentrations were normal in subjects with chronic renal failure when using the glucose specific oxygen rate glucose oxidase method. However, Weisinger et al. also showed that all patients with chronic renal failure have chemical diabetes by the criteria of Fajans and Conn (370), if plasma glucose is measured by the alkaline ferricyanide method and even the relatively specific 3-D-glucose oxidase-peroxidase method (which is subject to potential peroxidase reaction inhibitors, such as uric acid, ascorbic acid and other reducing substances). This finding is in agreement with ours. However, Weisinger et al. also showed that oral glucose tolerance is impaired and correlates with the severity of azotemia while our findings in the 4 to 6 hour post-absorptive state show that sub-totally nephrectomized rats probably cannot utilize endogenous sources of glucose; in fact they die if fasted for more than six hours. This is, perhaps in line with the

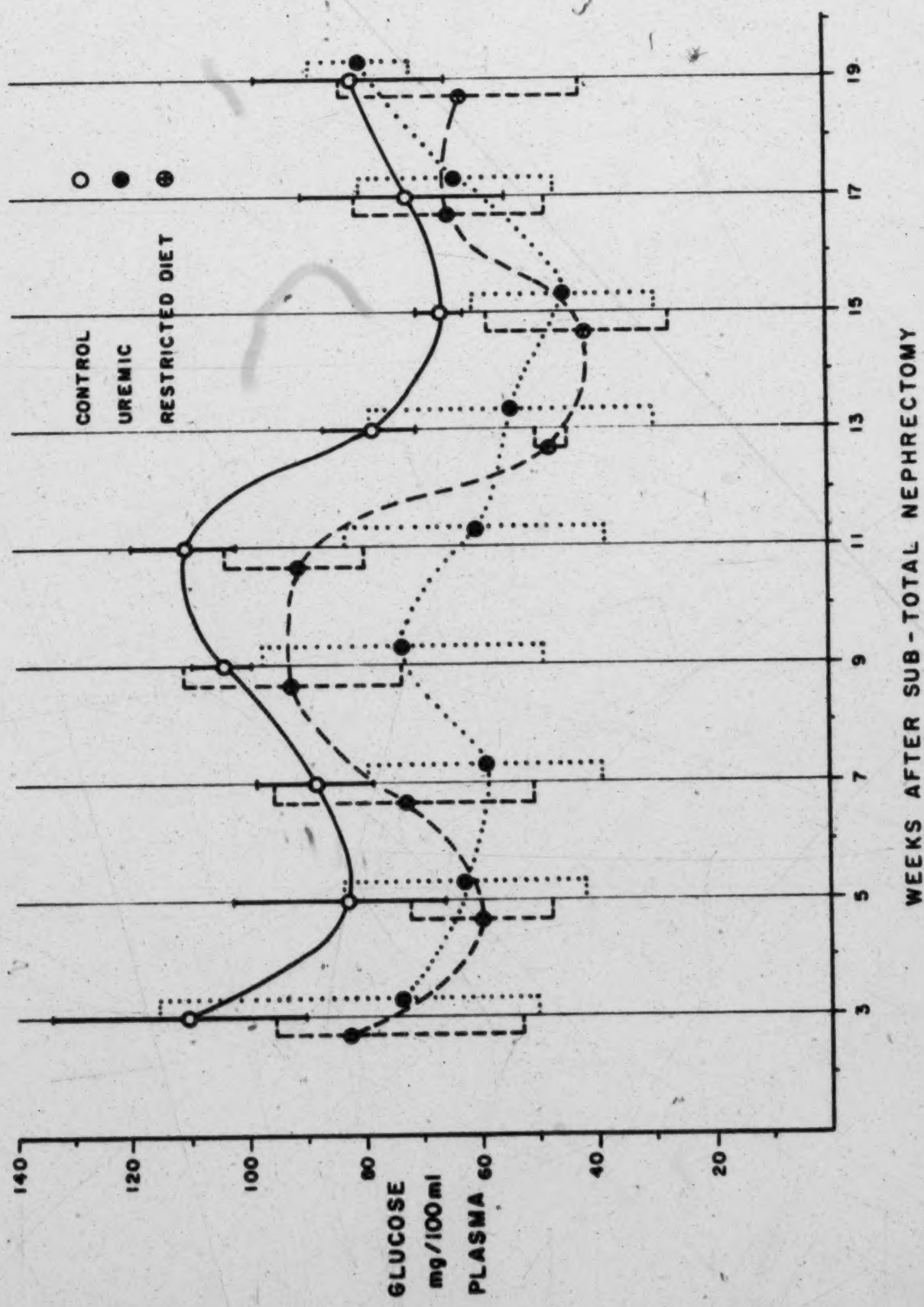
Weisinger findings that hemodialysis did not correct the glucose intolerance in the patients they studied. Cohen et al. (66) described the tendency to hypoglycemia to be a result of both the hepatic and peripheral inhibition of glucose uptake due to competitive blockage of the phosphorylating enzyme, by accumulation of related metabolites in uremia. Cohen et al. (289) re-examined this hypoglycemia tendency in subjects with renal failure using epinephrine and glucogen and insulin tolerance tests; observed responses to both tests showed a diminished glycolysis suggesting a deficiency in glycogen storage. They also implicated retained guanides (methyl guanidines and guanidine) to be agents effective in reducing liver glycogen. Shafrir et al. (302) also observed hypoinsulinemia, decreased pancreatic insulin content and decreased serum corticosterone levels associated with some what decreased glucose levels in their amino nucleoside induced nephrotic rats. It is also known that the body, in fasting, reduces gluconeogenesis to minimize protein breakdown. The hypoglycemic pattern observed in uremic rat is similar to that observed in restricted diet rats. This probably means that gluconeogenesis is reduced in sub-totally nephrectomized rats. If this is true, then one should expect increases in concentrations of plasma glucogenic amino acids and metabolites of the TCA cycle. As a fact, we did observe increases in the concentration of glucogenic amino acids in uremic rat plasma (Table XXV) and a decrease of SGOT, and increased BUN. This also indicates an increase in the concentration of oxaloacetate and aspartate and other glucogenic amino acids.

It is therefore expected that hypoglycemia may be due to either of one or more than one of the following causes:

(a) glucosuria, (i.e. spilling glucose in urine).
(b) restricted gluconeogenesis.
(c) increased glycolysis or conversion of G-6-P via the pentose monophosphate shunt.
(d) defective liver glycogen storage and release.
(e) increased lipogenesis and conversion into non-essential amino acids.

These have been examined to some extent, and are discussed in course under the respective parameters, namely urinary glucose level, glycogen isolation and structure determination, ^{14}C-glucose in vivo oxidation, G-6-PDH determination, glycogen cycle enzymes assay, ^{14}C-glucose in vivo conversion to lipid, glycogen and protein, and the assay of plasma free amino acids.

Fig. 34. Effect of sub-total nephrectomy and restricted diet on plasma glucose.


CONTROL ○
UREMIC ●
RESTRICTED DIET ⊕
GLUCOSE mg/100ml PLASMA
140
120
100
80
60
40
20
3
5
7
9
11
13
15
17
19
WEEKS AFTER SUB-TOTAL NEPHRECTOMY

7. Urinary Glucose Levels in Uremic, Sham Operated and Restricted Diet Rats (Table X)

Urinary glucose levels in uremic rats (for 3 consecutive 24 hour periods after 19 weeks of nephrectomy) were 12±3 mg% to 24±4 mg% which is much lower than the correspnding values of 15±1 to 141±25 mg% in RD rats and 26±2 to 103±17 mg% in SO rat urine. The corresponding plasma glucose in uremic, restricted diet and sham operated rats are 45±16 mg%, 41±15 mg% and 72±17 mg%, respectively (Table VI). This indicates that in the uremic the plasma glucose level is below normal and the urine glucose level is too low to be positive. This confirms that hypoglycemia in the uremics is not due to spilling of glucose in the urine but due to an abnormality of glucose metabolism. Our findings are contrary to those of Himsworth _et al_. who observed that in advanced bilateral renal disease, reabsorption of glucose is reduced in consequence of increased tubular urine flow and enlargement of the tubular diameter in the residual functioning nephrons (Abstract #944 in 185).

In RD rats low plasma glucose and a positive urine test suggest that spilling of glucose in the urine is one of the factors responsible for low plasma glucose levels, in addition to reduced gluconeogenesis. In SO rats, the normal plasma glucose levels and the positive urine test indicate a lowered renal threshold (372). However, in all three groups the combisitix tests were negative for urine glucose.

Urinary protein output was higher in uremics (300 mg%) and RD rats (300 mg%) than in SO rats (100 mg%). This indicates an increased concentration in the glomerular filtrate, decreased reabsorption or its diffusion from damaged tubular cells into the urine in the case of uremics and tissue metabolism in case of RD rats. Protein excretion is less in tubular than a glomerular damage (57).

Urinary pH was 9.00 in all three experimental groups (e.g. U, RD and SO). An alkaline urine is normally excreted if there is an excess of base or alkali in the body, if there is bacterial conversion of urea to ammonia or urinary tract infection or if the diet contains an excess of alkali (372). The normal rats excretion of alkaline urine may be due to one of these causes.

In tubular acidosis the kidney loses its ability to excrete an acid urine. The excretion of ammonia is reduced and the urine contains bicarbonate, probably due to deficiency of carbonic anhydrase. This might be the reason for the alkaline pH seen in the uremic rats (76). However, urinary pH also measures the distal tubular function. However, alkalosis as shown by urinary pH in uremia is in agreement with hypoglycemia as observed in uremic rats. Acidosis causes rapid glycogenolysis with glucosuria and hyperglycemia, both of which are absent in our uremics (227,228).

Urine volume over the period of study was always much larger in uremic rats (17 ml-81 ml) than those in RD (2 ml-14 ml) and SO (3 ml-8.5 ml). The polyuria as seen in uremics is in line with what would be expected. RD rats show oliguria which is in line with the output of urinary protein associated with starvation. The overall effect in uremia seems to be primarily due to the effect of sub-total nephrectomy (i.e. urine volume is increased or decreased suggesting isosthenuria) (372).

8. The Effect of Physical Forms of Food on SGOT, Serum CPK, and Other Related Parameters in U, RD and SO Rats (Table XI)

To standardize the diet restricted rats, the necessity for determination of the minimum food intake of the uremic rats arose. This was done by giving uremic rats a measured quantity of powdered diet daily over a period of 3 to 4 weeks. The minimum food intake of the uremic rats (≈23 g/day/rat) was thus determined and this amount was given constantly to the restricted diet group to maintain partial starvation. Therefore, this also necessitated the comparison of the effect of pellet and powdered forms of food on U, RD and SO rats. All experimental groups thereafter were maintained on pellet diet. This table shows no significant difference, with respect to the physical form of the diet, in any of the biochemical parameters, namely, SGOT, alkaline phosphatase, glucose and BUN studied over a period of 15 weeks. This also suggests that the absorption of food in U, RD and SO is not affected by the form in which it is given. This supports the findings of others (291).

9. *In Vivo* Oxidation of Intraperitoneally Injected ^{14}C-Glucose by U and SO Rats (Table VIII)

The T_m (time of maximum rate in min) for CO_2 exhalation in sub-totally nephrectomized rats was nearly half (46-50%) that required by the SO rats, when C-1 labelled glucose was administered; with U-^{14}C-glucose administration the T_m required in U rats was 0 to 42%. The rate of conversion (as a % of the dose) of ^{14}C-1-glucose and ^{14}C-U-glucose to $^{14}CO_2$ by U rats was significant irrespective of the position of labelling. The total conversion was *ca*. 74% (the range being 3.7 to 7.9% of the dose in U rats and 0.5 to 2.9% of the dose in SO rats) higher in the U rats than the SO rats for ^{14}C-1-glucose, and 170% (the range being 2.9 to 9.6% of the dose for U rats and 1.7 to 3.3% of the dose for SO rats) higher in U rats for ^{14}C-U-glucose.

Acceleration of glucose oxidation (179) and inhibition of oxidative phosphorylation in uremia is well known (182). It has been suggested by Bloom *et al*. (378) that CO_2 will be derived from C-1 glucose exclusively if and only if the hexose-P regenerated by the cycle is constrained from oxidation in favour of the hexose-P added originally, but if removed then CO_2 can be obtained from other positions by rearrangement. Our data supports the latter possibility, and also implicates the pentose monophosphate shunt as being more effective in uremics. The accomplishment of this pathway is not oxidation of glucose as an end itself but rather its conversion to precursors as a means for the biosynthesis of

i. pentose

ii. nucleotides and derivatives, co-enzymes, polysaccharides,

iii. fatty acids, steroids, etc., and

iv. NADPH, a source of reducing power for these biosynthetic routes. These data also suggest the possibility of increased activity of pentose monophosphate shunt enzymes in the uremic condition. They suggest as well that glucose handling in uremic rats proceeds more through the oxidative pathway, which is the major source of CO_2 production.

10. Hepatic Glycogen Structure in Uremia (Table IX)

The structural analysis of hepatic glycogen obtained from 4-1/2 week postoperative uremic rats showed significant differences in terms of:

i. the number of non-reducing ends (67% higher) and 1,6-bonds (branching points, 70% higher)

ii. the number of segments per mole (68% more) and

iii. the number of glucose residues per segment (50% less). Electron microscopy of hepatic tissues of U rats reflected the changes as an abnormal deposition of glycogen (fig. 27). However, surprisingly, the molecular weight of glycogen from both sources showed no significant differences, suggested a change in the intra-molecular structural arrangement, and possibly irregularities in glycogen cycle enzymes, namely, synthetase, phosphorylase, and the branching enzyme, amylo-1,6-glucosidase.

The increase in the number of hepatic glycogen molecular segments (by 68%) along with a decrease in the number of glucose residues per segment (by about 50%) in uremia probably suggests that the tendency to branching is more than towards the proportionate lengthening of the structure, due to the competitive influence of the branching enzyme over the chain lengthening enzyme. This would be due to the change in the equilibrium in the milieu, in the uremic environments (1,21,100,103,104,112,122,123,169). Phosphorylase, an enzyme of dual function, adds glucose residues to 1,4-glucose chain and synthetase catalyzes only formation of α-1,4-bonds; the branching enzyme acts upon these chains to convert some of the 1,4-linkages to 1,6-

linkages, forming branching (238).

The muscle enzymes, unlike the liver glycogen cycle enzymes, are under separate gene control. The increase in branching in the uremic hepatic glycogen suggests an alteration in the normal activity of amylo-1,6-glucosidase towards increasing conversion of 1,4-linkages to 1,6-linkages compared to normal rat glycogen. Branching enzymes and phosphorylase are known to be more active in large white fibres, while UDP-glucosyl transferase activity is concentrated in the small red fibres of rat skeletal muscle (245), but we do not know the partition concentration or compartmentalization of these enzymes in the liver. It is perhaps, this alteration in compartmentalization in the uremic liver (of these glycogen cycle enzymes) which is responsible for the differential structures. This yet has to be explored; however, it is the "specificity" of this "branching enzyme" which determines the inter-branch distance along the glycogen chain (246,238).

11. Hepatic Glycogen Content and the Corresponding Biochemical Parameters in Uremia (Tables XII and XIII)

Hepatic glycogen content in uremia over a period of 1 to 19 weeks shows 5.5±2.6 mg% ($P \leq 0.025$) in contrast to SO rats (3.9 mg% 1.6); hepatic glycogen content in RD rats was not significantly different from SO controls. Periodic study shows that there is a tendency in the uremic liver for abnormal deposition of glycogen. These biochemical findings though statistically insignificant ($P<0.01$ to 0.40), are nonetheless, supported by electron microscopy (see figs. 27,28, and 29) in many cases.

The electron microscopy reveals the glycogen deposition in the liver in the same decreasing order of U, RD and SO rats as seen by biochemical isolation and gravimetric determination. The abnormality of increasing deposition is shown to a greater degree in the 1 to 9 week than 10 to 19 week post-operative period.

The rate of intestinal absorption, and metabolism of absorbed substances, protein diet, and physiological changes are known to control tissue glycogen formation. For example, in prolonged starvation, because of loss of the capacity to oxidize glucose, hepatic glycogen is more prone to conversion.

Therefore, in fasted animals or in aminals in the uremic condition, where loss of appatite or fasting is a secondary aspect, one should expect to have a better gluconeogenesis from tissue protein to yield the larger quantity of glucose to tissues.

However, the higher liver glycogen content and low plasma sugar in both uremic and restricted diet rats seem to be paradoxical. The maintenance of high liver glycogen, in the starvation or semi-starvation condition of RD and U rats, is only possible by the tissue protein effect or a high protein diet. As will be shown later in scrutiny of individual cases, the levels of the individual glycogen cycle enzyme activities are compatible with the corresponding species liver glycogen content. This is in line with the general agreement that there is a relationship between the glycogen content of the tissues and respective tissue glycogen cycle enzyme activities (245).

However, our results suggest that there is abnormal deposition of hepatic glycogen in the uremic state. The periodic tendency of restoring hepatic glycogen levels to normal, reflects the endeaver of the animal (made through homeostasis)to recover from the uremic effects without much permanent success.

Our findings of abnormal glycogen deposition in uremic liver are largely compatible with our findings of glycogen cycle enzymic activities in most of the periods studied, but are in contradiction with the findings of Cohen _et al_. (289) that diminished hepatic glycogen stores are secondary to glycogen synthesis or release. Dzurick _et al_. (290) and Boucot _et al_. (291) demonstrated normal liver glycogen content in uremic patients and in acutely and chronically uremic rats.

Our findings were also confirmed by _in vivo_ conversion of ^{14}C-glucose to glycogen in uremic, sham operated and restricted diet rats (Tables XXII and XXIII).

Uremic hepatic glycogen stores on a liver mass basis were highest, followed by the RD and SO groups (Table XXII). The distribution of ^{14}C-glucose label in glycogen on a per cent of liver basis was highest in RD, followed by SO and U, but when levels are expressed per 100 g of liver glycogen the appearance of ^{14}C-glucose label was second highest in U followed by SO and preceded by RD (Table XXIII). Isolation of hepatic glycogen was done by the gravimetric method of Bloom _et al_. (331) who reported more than 80% extraction without causing alteration of native molecular state, unlike classical Pflüer methods.

Since March 21, 1857 when Claude Bernard presented to the Societe de Biologie in Paris the report of isolation of glycogen from liver, solubilizing of tissue in hot alkali has been used to extract glycogen from tissue. The glycogen precipitatedby ethanol from the alkali extracts contain certain foreign materials which interfere with quantitative estimation of the carbohydrate (231,232) but glycogen precipitated from cold TCA is completely free from interferring foreign materials; also, unlike the drastic alklai extraction, its native molecular structure remains intact. The extract ('TCA supernatant') is designated as the "free" form (233,17); an extracted "fixed" glycogen remains with TCA precipitate and can be extracted with hot alkali. Some investigators used this method to differentiate two glycogen types while others made differentation by extracting with cold and hot TCA (383,384) (cold TCA extract being "free" and hot TCA extract "fixed" forms) to substitute for the hot alkali extraction method. Some claim (381,385) the "fixed" fraction to be more active while others found the "free" form to be metabolically more active (386,387). Meyer and Zalta (218) do not subscribe to these views and to this separation into two forms of glycogen. Carole et al. (380) claim that the material measured as fixed is not glycogen, as chemically and chromatographically it behaved as a polymer of glucose similar to glycogen (219) and the enzymatic degradation of the "fixed" fraction did not differentiate it from the "free"form (388). Hanson et al. (389) extracted 87% of liver glycogen while Bloom et al. (331) extracted 85% of liver glycogen by using cold 10% TCA method. The use of the cold 10% TCA method of Bloom et al. (331) was based upon this rationale.

Biochemical parameters and hepatic glycogen content

The range of BUN (68±18 to 141±30) in uremic rats does not seem to have any quantitative effect on the glycogen content and the corresponding SGOT, serum CPK, plasma, alkaline phosphatase activities and plasma glucose levels.

12. Percent Autolysis (at 25°C) of Hepatic Glycogen in U, SO, and RD Rats (Table XIV)

Exposure of liver obtained from U, SO and RD rats 19 weeks post-operatively, at 25°C for half an hour, showed that uremic liver underwent no detectable loss of glycogen while loss of glycogen in SO and RD under the same condition was 41±2% ($P \leq 0.20$) and 31±9% ($P \leq 0.25$), respectively. Autolysis for a longer period (90 minutes) of exposure resulted in a 36% loss of uremic hepatic glycogen ($P \leq 0.10$) with corresponding losses of 58% and 41% ($P \leq 0.25$) for SO and RD rat hepatic glycogen, respectively.

Autolysis is a combined and concerted action of amylases (α and β), phosphorylases (a and b) and amylo-1,6-glucosidase. α-amylase causes random hydrolysis of the α-D(1→4) linkage in both exterior and interior glycogen chains, giving maltose (220). β-amylase causes a stepwise catalysis of the alternate linkages in an α-(1→4) linked D-glucose residues, liberating maltose (221); it starts at the non-reducing end of the chain and proceeds until 1,6-amylo-glucosidic linkage is reached. Thus, it is confined to the exterior portions of the chain, producing the high molecular weight limit dextrin (β-dextrin), or maltose, containing all the inter-chain linkages.

Phosphorylase (222) in the presence of P_i removes D-glucose residues from the exterior chain of glycogen, producing G-1-P by phosphorolysis of the 1,4-glucosidic linkage, in single steps; it cannot by-pass inter-chain linkages. Amylo-1,6-glucosidase then hydrolyses 1,6-glucosidic bonds of the β-limit dextrin (224) to release glucose.

Our results showed that all of these enzymes in uremic liver are almost inactive in the first 30 minutes, to and become only 40% active in the 90 minutes period compared to SO and RD controls. The reason is hard to ascribe. This effect may also explain why uremic rats cannot tolerate withdrawal of food for more than 4 to 6 hours. This paradox seems to be in line with the findings of Hugchings et al. (265) who explained the cause of glucose intolerance, in long term intermittent dialysis patients, to result from a slowed rate of glycogenolysis. However, our findings at least partially account for the hypoglycemia as observed in sub-totally nephrectomized rats.

13. Glycogen Cycle Enzymes and the Pentose Monophosphate Shunt Enzyme

(a) Muscle and Liver Amylo-1,6-Glucosidase Activities in U, RD and SO (Table XV and XVI)

In uremic muscle, branching enzyme activity in the 1 to 9 week post-operative period shows no change (3%, P≤0.495) by Hers units, and a decrease (27%; P≤0.10) in terms of μM glucose per g tissue per hour, whereas in the second post-operative period of 10 to 19 weeks, activity was significantly decreased (by 41 to 59% P≤0.0025 to 0.05) when compared to SO. In RD rat muscle there is a 29% increase (P≤0.15) in the first period and 15 to 17% decrease (P≤0.30) in the second period compared to control, both of which are insignificant.

Compared to muscle activity, amylo-1,6-glucosidase activity in uremic liver was about 12% higher in the first 1 to 9 week post-operative period and 25% lower in the second period (10 to 19 week post-operative period), where as in RD liver, it was lowered by 40% in the first period by 14% in the second period. This indicates that the decreased activity in uremic liver in the second period might be partly due to decreased food intake, in addition to the uremic effect.

The tendency for amylo-1,6-glucosidase activity to increase in the first period and to decrease in the second period in both U and RD muscle is very significant in that there might be a homeostatic effort to maintain the intramolecular structure of glycogen. Although an increase in amylo-glucosidase activity means an increase in branching, the effect may be counteracted by the activity of the chain lengthening and glucose adding enzyme phosphorylase, and or synthetase. However, the activity of glucosidase in U and RD liver shows the inverse effect (i.e. in U rats it increases in the first period with corresponding decrease in

RD followed by decrease in U with corresponding increase in RD in the second period). This indicates that in U rats the amylo-1,6-glucosidase activity is independent of semi-starvation, condition. When compared to the findings of hepatic glycogen structure in the uremic rat, this activity correlates very well (i.e. in the same period, a 17% increase of amylo-1,6-glucosidase activity caused about a 70% increase of branching points with 68% increment of segments and 67% increment of non-reducing ends in the glycogen structure (Table IX). This is confirmed by the fact that in the same period, that glucose residues per segment in the uremic hepatic glycogen molecule (Table IX) decreased by 44%, the synthetase activity (which adds glucose to the chain) shows a decrease of 25-66% (P_0.005 to 0.05) (Table XVIII). Again, the periodic change of glycogen stores in uremic rats correlates very well with the decrease of synthetase activity and phosphorylase activity.

(b) Muscle and Liver Synthetase 'I' and 'D' Activities (Table XVII and XVIII)

Uremic muscle has twice the amount of synthetase 'I' and almost three times the amount of synthetase D activity as the liver in the first period, while in the second period muscle synthetase 'I' activity equals that of the liver, although muscle synthetase 'D' activity is still twice as much as liver synthetase 'D' activity. In the muscle of RD rats the same activity of synthetase 'I' is observed as in the liver in both periods, but synthetase'D'activity in RD muscle is almost double compared to those in liver in both periods. This shows that the synthetase activity in muscle of both U and RD rats is much higher than the activity in liver.

Compared to SO controls, uremic muscle synthetase 'I' is increased by 170% ($P \leq 0.01$) in first period and marginally increased (14% $P \leq 0.25$) in the second period while the corresponding figure in uremic liver is quite opposite (i.e. decreased by 25% ($P \leq 0.05$) and 6% ($P \leq 0.005$)) in the second period. The restricted diet rat liver synthetase 'I' activity shows a decrease to the same extent as that of uremic liver in the first period but in the second period RD liver enzyme decreased by a factor of 3 times that of U liver enzyme. This suggests that perhaps in the first period the uremic effect on enzyme was partly due to the semi-starvation condition, but in the second period the effect entirely due to uremia.

Synthetase 'D' is decreased by 66% and 18% (about 1/4 of the first period effect) in the uremic rat liver in first and second period, respectively, which in restricted diet rat liver it is decreased by 35% and 17% (1/2 of the first period effect) in first and second period, respectively compared to control. This suggests that the effect in the enzyme is partly due to uremia and partly due to semi-starvation in the first period while in second period the effect seems to be due to more starvation.

Compared to synthetase 'D' activity, the activity of synthetase 'I' is much higher in both uremic muscle and liver, suggesting that G-6-P in uremia is either depleted or very limited. Consequently, synthetase 'D' is inactive in uremia irrespective of first and second periods. However, the opposite is true for RD synthetase in both muscle and liver (i.e. in RD rats, synthetase 'D' is more active than 'I', meaning more availability of G-6-P). The increased activity of G-6-PDH in uremia, in addition to other factors, may well limit the G-6-P. Free UDP, which accumulates if the supply of both ATP and glucose is limited, inhibits the phosphoenzyme (synthetase "D") strongly glycogen synthetase phosphatase converts "D" (phosphoenzyme) to "I" (dephosphoenzyme), and is inhibited by glycogen itself. Consequently, if glycogen is depleted, the "I" form dominates (as seen in uremic muscle and to a lesser extent in uremic liver, Table XVII and XVIII), as the glycogen concentration increases, the "D" form increases. Again, the diminished glycogen concentration releases glucagon from the pancreas and epinephrine from adrenal medulla, causing glycogenolysis. Insulin does the opposite; it enhances glycogen synthetase activity (i.e. insulin insufficiency should decrease glycogen synthetase activity and consequently liver glycogen formation) increases the operation of phosphogluconate pathway to provide NADPH for increased fatty acid synthesis (247,213). Insulin administration usually increases muscle glycogen but not liver glycogen, and oxygen is necessary for glycogen synthesis. Also, glucocorticoids increase gluconeogenesis, and tend to increase liver glycogen by augmenting the supply of glucose from non-carbohydrate precursors; this favours accumulation of liver glycogen. Excessive thyroid hormone mobilize and cause disappearance of liver glycogen (213,247).

However, we have observed that glycogen formation in the uremic liver is not decreased. On the contrary, muscle synthetase activity has been shown to be increased by 170% in our uremic rats although in uremic rat liver, it shows a tendency towards decrease and uremic plasma shows hypoglycemia and increased liver G-6-PDH activity (by which no ATP is supplied). All of these favour the idea that insulin insufficiency may not occur in sub-total nephrectomy. Available data suggest that in nephrectomy there is a prolongedhalf life of insulin (254), and that hyperinsulinemia in uremia does occur (265,275,276).

(c) Muscle and Liver α-Glucan Phosphorylase 'a' and 'b' Activities (Tables XIX and XX)

In uremia muscle total phosphorylase activity, expressed as the ratio of 'a' to 'a+b', is decreased by 41% (Table XIX) in first period of 1 to 9 weeks and by 12% in the second period of 10 to 19 weeks, while in the uremic liver it is doubled in the first period and shows no change in the second period. In RD muscle, the ratio is decreased by 60% in the first period and by 40% in the second period, while in RD liver it shows the opposite result, increasing by 42% in the first period and showing no change in the second period. This suggests that the effect of uremia on this enzyme may be an accumulative effect of uremia and starvation. Both the activity of the 'a' form (independent of AMP) and the 'b' form (AMP dependent) were the same in uremic muscle in the first period, but in the second period the 'b' form was doubled, indicating an increased concentration of AMP. However, the latter effect prevails in the RD animals (i.e. the 'b' form activity is 2 to 3 times the 'a' form activity) suggesting that in restricted diet, accumulation of AMP might have triggered this phenomenon. However, in SO, the 'b' form becomes twice as active as the 'a' form only in second period, suggesting the normal activity of phosphorylase in U and RD muscle.

In contrast to the SO control, the activity of 'a' in both uremic liver and RD liver have been shown to be twice the activity of the 'b' form, suggesting that perhaps AMP availability in the liver has been limited by the semi-starvation condition. This analog might explain the ready reversibility of the phosphorylase enzyme system for synthesis as well as for the breakdown of glycogen. This also reflects the fluctuating concentration of glucagon and epinephrine, the two 'insulin antagonists'.

An increase in the concentration of AMP, and a decrease in the concentration ATP and G-6-P stimulates operation of phosphorylase 'b'. This is the common rule. Our findings of liver enzymes show that G-6-PDH (as will be discussed later) increases and the phosphoenzyme ('D' synthetase) decreases in uremia. Therefore, naturally, the operation of phosphorylase 'b' should be expected to be increased, but which instead is decreased in both periods by almost half of 'a' form. This paradox suggests that perhaps glycogen in the liver is abundant; in its presence phosphorylase 'b' is ultimately converted to phosphorylase 'a'.

The abnormal glycogen storage, the low amylo-1,6-glucosidase activity, the decreased glycogen synthetase and the high ratio of total phosphorylase activity suggest that perhaps by reversal of the phosphorylase pathway, glycogen synthesis is taking place in uremia.

Amylo-1,6-glucosidase, an alternative pathway for glycogen synthesis (228) from glucose, circumvent the HK,PGM,phosphorylase sequence. Although in this pathway glucose is a better precursor for glycogen synthesis than are G-6-P G-1-P, this pathway is ATP dependent and is an ATP-generating system. Perhaps this is why amylo-1,6-

glucosidase, which is increased in the first periods, decreases in the second period of chronic uremia, in which case glycogen synthetase and phosphorylase take over (Table XV to XX) utilizing G-6-P via UDP-G and G-1-P, respectively.

(d) Liver G-6-PDH Activity
(Table XXI)

A 65% ($P \leq 0.005$) activation of liver G-6-PDH occurs in uremia in the 1 to 9 week period; 63% activation ($P \leq 0.0005$) occurs in the second post-operative period, as compared to the SO liver enzyme. In contrast, RD liver enzyme shows 0.73% activation ($P \leq 0.49$) in the first period and 18% ($P \leq 0.20$) in the second period, both of which are statistically insignificant.

These results clearly show the effect of uremia alone on the enzyme. Maer *et al*. (155) demonstrated no effect on G-6-PDH 24 hours after bilateral nephrectomy, although they did see a decrease in HK, GK and PFK. However, our results suggest that there should not be any effect (due to sub-total nephrectomy) on the glycolytic pathway up to the G-6-P production stage (i.e. HK and GK). This is supported by the work of others (175). The red blood cell enzyme G-6-PDH is known to be increased in uremic sera (185) whereas the erythrocyte enzyme transketolase, which is thiamine pyrophosphosphate dependent, is reported to be depressed by uremic plasma (177).

The pentose monophosphate shunt pathway is an oxidative pathway, second only to the non-anaerobic glycolytic pathway. By this pathway, total combustion of glucose occurs independently of TCA cycle. If there is any Pasteur effect (i.e. inhibition of glycolysis by oxygen) beyond G-6-P due to uremia then the only obvious way of glucose combustion is to shunt G-6-P to the Pentose monophosphate pathway; this would take care of the combustion of glucose. This hexose monophosphate shunt is under the control of a series of enzymes; G-6-PDH, isomerases, transketolases, and transaldolase. All of these reactions interact together,leading to the oxidative conversion of glucose either to triose phosphate or to CO_2. The net reaction is:

$$C_6H_{12}O_6 + 6O_2 + \leq 36\ ADP + \leq 36\ P_i \rightarrow 6\ CO_2 + 6\ H_2O + \leq 36\ ATP$$

In other words, $6\ \text{glucose-6-P} + 12\ NADP \rightarrow 5\ \text{G-6-P} + 6\ CO_2 + 12\ NADPH + 12\ H^+ + P_i$

or, $6\ \text{G-6-P} + 6\ O_2 \rightarrow 5\ \text{G-6-P} + 6\ CO_2 + 6\ H_2O + P_i$

This pathway of oxidation of glucose is not an end in itself but rather a means to convert glucose to precursors for the biosynthesis of pentose, nucleotides, coenzymes, polysaccharides, fatty acids, steroids and NADPH (a reducing power for these biosynthetic ranks). Therefore, it is quite in line with the biological demands which arise when an animal is under stress of uremia, for this pathway to operate to meet the animals' requirements for homeostasis.

14. *In Vivo* Conversion of ^{14}C-U-D-Glucose 12 Hours After IP Injection into Protein, Glycogen, Lipid and Fatty Acids by Uremic, Sham Operated and Restricted Diet Rats (Table XXII, XXIII, and XXIV)

(a) Tissues Studied

The constituents of various tissues were assayed to compare their component distributions in U rats with those in RD and SO.

i. Kidney

The residual renal mass (1/3 of the single remaining kidney) in uremic rats expanded perhaps in an attempt to compensate the nephrone function. It was equal in weight to the single kidneys of RD and SO rats.

ii. Liver

The mean total liver mass of the uremic rat was 25% greater than the total liver mass of RD and SO rats. This will explain why the total glycogen content of uremic liver on a mass basis is higher than those in RD and SO, although on a per gram basis the picture looks quite different.

(b) Tissue Components

i. Lipid

The distribution of lipid in the liver is highest in SO rats, followed by [illegible] ($P \leq 0.025$) and RD rats ($P \leq 0.025$), and in adipose tissue is again highest in SO rats but followed by RD ($P \leq 0.01$) and then U rats ($P \leq 0.05$). However, the distribution of lipid in muscle is highest in RD rats ($P \leq 0.05$) and lowest in SO rats placing U rats ($P \leq 0.0005$) next to the RD group. These observations are in line with the findings obtained earlier, in that uremic and RD liver show more deposition of glycogen and that their muscular proteins utilization is also associated with the semi-starvation condition. Paradoxically, the plasma free fatty acid level is highest in uremia, suggesting perhaps a degree of lipolysis of adipose and muscle lipids to meet the body demand to inhibit their own biosynthesis by feed-back inhibition. In glucose metabolism, FFA is an end product and is capable of selectively inhibiting, in a dose dependent fashion, the key hepatic enzymes of glycolysis (GK, PFK and PK and lactate production from glucose). Our findings on G-6-PDH activity suggest no such inhibition on GK, but below the G-6-P stage there seems to be some inhibition so that G-6-P is channelled through the pentose monophosphate shunt. It is likely that serum FFA may have some inhibitory effect on glycolytic enzymes below G-6-P (e.g. PFK and PK).

Roodvoets *et al*. (189) observed hyperlipemia in chronic renal failure characterized by an increase in the pre-β-lipoprotein, the major lipid fraction of which consists of lipids, and triglyceride (201). Triglyceride, however, is of endogenous origin being synthesized mainly in liver (201). Frederickson *et al*. (201) found that plasma lipid accumulation constitutes a type IV hyperlipoproteinemia, typically carbohydrate inducible.

High plasma FFA may be due to either high activity of lipase or to slow disposal of plasma fatty acids due to uremic disturbances. In nephrotic rats, liver metabolite patterns (for example accumulation of α-glycerophosphate) is conducive to lipogenesis, as α-glycerophosphate enhances fatty acid synthesis (204). It has been recognized that increased glycolysis is associated with lipogenesis or increased gluconeogenesis, amino acid metabolism and enhanced fat utilization. Findings of increased plasma FFA, G-6-PDH and muscle lipid in uremia support this contention in part. Conversion of ^{14}C-glucose into lipids of muscle, adipose tissue and liver is highest in RD rats, followed by uremic rats. In uremia, the conversion of ^{14}C-glucose into lipid are 84% in muscle ($P \leq 0.0025$); 562% in adipose ($P \leq 0.0005$) and 52% in liver ($P \leq 0.005$), as compared to SO. In plasma FFA formation from ^{14}C-glucose, the uremia was the lowest of the three groups, having undergone a 71% decrease ($P \leq 0.0005$) as compared to SO. This suggests that the dose of ^{14}C-glucose was not enough to produce FFA to such extent as to show feed-back inhibition of its own biosynthesis, in uremia (which may have different biogenic pattern than RD or SO). The pools of radioactive lipid in adipose tissue, liver and muscle were not sufficient in uremic rats to contribute to labeled plasma FFA by lipogenesis. However, this points out that there is a different pattern of plasma FFA lipogenesis in uremia

It has been suggested that hyperlipogenesis in nephrotic rats may or may not occur, depending on the manner of induction of nephrosis, the severity of the disease and above all on the synthesis of apolipoprotein. If lipogenesis is not increased once the apolipoprotein is synthesized the apolipoprotein may draw its lipid component from preformed liver lipids and produce a shift in plasma liver lipid distribution without greatly changing their pool size (176). The large pool size of labeled adipose lipid in our experiment supports the contention of others that glucose is a good precursor of triglyceride (135). The ^{14}C pool size is smaller in liver than in muscle than in adipose tissue. This also supports other workers who showed increased hyperlipidemia, and hepatic accumulation of triglycerides, cholesterol and low density lipoproteins (206,207,168). The fall of plasma ^{14}C-labeled FFA observed in our uremic group after ^{14}C-glucose injection is in agreement with the findings of Losowsky _et al_. (190), who reported a fall of FFA after IV injection of glucose.

Endogenous triglycerides are synthesized by intestine and liver cells, which after synthesis, secrete into the circulation. The one major mechanism for removal of lipid is via lipoprotein lipase activity, predominantly in adipose tissue (390). Increased endogenous synthesis reflects the involvement of elevated circulating insulin levels (394,395). This increased level of ^{14}C-labelled lipid in muscle, liver and adipose tissue in the nephrotic rats once again suggests as to the increased synthetase activity and G-6-P DH activity, that insulin levels might be elevated. This disorder of lipid metabolism may possibly be due to carbohydrate induction of the lipoprotein abnormalities; suggested mechanism involves impaired removal due to a decrease in post heparin lipolytic activity and elevated insulin levels which stimulate hepatic triglyceride synthesis (391,392,393). The increased deposition of labelled adipose lipid with a low level of labelled plasma FFA are in line with the hypothesis of triglyceride synthesis and removal. Triglycerides are normally formed by simple esterification of both circulating fatty acids from adipose tissue stores and newly produced fatty acids derived from carbohydrate (lipogenesis). Lipogenesis requires insulin; the compensatory hyperinsulinism associated with the uremic state plays a role in the alteration of triglyceride transport. Cholesterol, phospholipid and triglyceride, three major lipid classes, are transported while linked to protein in soluble molecular aggregates. The important and major fraction of the lipid transport protein is the triglyceride rich, very low density lipoprotein ("pre-β" or α_2 migrating in addition to albumin bound free fatty acids) which is known to be consistently elevated in uremia. These lipoprotein abnormalities have been widely recognized to be a consequence of the protein urea associated with the nephrotic syndrome (396). The proteinuria observed in the nephrectomized rats (Table X) supports this contention.

ii. Protein

Liver glycoprotein (TCA precipitate) elevation was highest in RD rats (192%) and then in the U rat (26%) as compared to the SO control group ($P-0.0005$). Plasma protein (TCA precipitate) in the RD group decreased by 55% ($P \leq 0.01$) and in the U rats by 45% ($P \leq 0.025$), indicating that elevated synthesis of protein was perhaps either abnormal protein or incomplete protein due to non-sense coding as a consequence of the cumulative effects of malnutrition and uremia, or due to the conversion of glucose into lipids as a consequence to the elabbration of lipoprotein peptides (128,129). Urinary loss of plasma protein as seen in Table X may be responsible for the institution of compensatory liver protein synthesis and the tissue hyperlipidemia seems to be related to the severity of protein urea and perhaps to the rate of protein renewal. This view has been shared by other workers who linked plasma protein levels to the extent of plasma lipid elevation (397). The plasma non-protein nitrogen (TCA supernatant) was highest in RD (elevated by 8%; P≤0.0125) and was decreased in uremia by 18% (P≤0.05) as compared to SO. This is quite in agreement with our other findings of elevated BUN in U and normal BUN in RD compared to SO rats (i.e. understandably most of NPN in uremia is channelled to urea synthesis). The conversion of ^{14}C-glucose to glycoprotein and the distribution of the label in liver protein (TCA precipitate) of U,RD and SO rats follow the same pattern in support of the findings (as above) of distributions of protein in U, RD and SO rats. That is, incorporation of ^{14}C into the rats resulted in the largest increase in specific activity of glycoprotein of RD (146% increase) (P≤0.0005) followed by U (28% increase P≤0.0025) as compared to SO. This is in agreement with findings of others (347,128,127).

iii. Glycogen

Liver glycogen distribution was highest in uremic rats (132% higher P≤0.0005) followed by RD (77% higher P≤0.0005) compared to SO. Incorporation of ^{14}C was on a whole liver mass highest in RD rats or per 100 g liver glycogen basis U as compared to the SO group.

The greater incorporation of ^{14}C into uremic hepatic glycogen (1.13×10^5 dpm/8.52% glycogen) than that in SO hepatic glycogen (5.40×10^5 dpm/3.7 g% glycogen) is in agreement with the work of others who observed that at the expense of incorporation into glycogen of all tissues and peripheral fat and protein, enhancement of conversion of glucose carbons into the proteins and lipids of the plasma and liver pool occurred (347). However, in consideration of the conversion of ^{14}C into lipid, protein, glycogen and FFA, the highest specific activity appearance of the ^{14}C-glucose in U rats was in glycoprotein followed by hepatic glycogen, liver lipid, muscle lipid and then adipose lipid. This was also true for [illegible] and SO rats. This means that in the tissues of the nephrotic rats there is a shift in the pattern of glucose utilization. Glucose, instead of being utilized for the synthesis of tissue glycogen and peripheral fat and protein alone, undergoes enhanced conversion into plasma and liver proteins, and liver and other tissue lipids. This preferential channeling of glucose to proteins and lipids only means that a large proportion of the glucose is being utilized to enhance their production.

These findings are in agreement with the concept of Marsh _et al_. (398,399) that in nephrosis, increased mobilization of peripheral carbohydrate, fat and protein reserves does occur.

(c) Distribution of ^{14}C-Glucose, as % Dose, in Tissue (Table XXIV

In uremic rats, kidney was the highest recipient of dose (0.16% P≤0.002) followed by muscle, liver, plasma;

adipose tissue was the lowest recipient. Conversion efficiency was highest in the liver, followed by adipose tissue. In RD rats, the liver was the highest recipient (0.18% $P \leq 0.0005$) followed by muscle, kidney and adipose, tissue; plasma FFA was the lowest, showing a pattern similar to the SO controls. This suggests that the uremic effect singles out other effects due to starvations in the channelling of ^{14}C-glucose into lipid, protein and glycogen is controlled by the efficiency of the tissue, and not by the concentration of activity as ^{14}C-glucose. Cummings et al. (292) reported incorporation of ^{14}C-glucose in CRF rat liver glycogen, and lipid, and $^{14}CO_2$ production as normal.

15. The Plasma Aminogram in Uremic, Restricted Diet and Sham Operated Control Rats (Table XXV and XXVI)

In the uremic condition, in view of the compensatory increase in plasma protein synthesis (as observed in our experimental data described above), the amino acid profile is very interesting. It is known that glucocorticoids induce hepatic gluconeogenesis from amino acids, and enhance mobilization of amino acid from extra hepatic proteins by increasing transport to the liver with the consequent increased levels of several amino acids both in liver and plasma (400,401). The liver synthetic activity, particularly increased transaminating and deaminating enzymes, draws amino acid from peripheral sources as evident from extensive muscle loss in uremia similar to that seen in glucocorticoid administration (400,401).

(a) Essential Vs Non-Essential Amino Acids

The total essential amino acid concentration in the plasma of nephrectomized rats shows a statistically significant decrease of 22 to 24% ($P \leq 0.0125$), with statistically insignificant elevation of a few ($P \leq 0.45$) over both periods of 1 to 9 and 10 to 19 weeks. The total non-essential amino acid concentrations in uremic rats show an increase of 13 to 55% ($P \leq 0.0125$) (except glutamic, cystine and prolin with $P \leq 0.15$ to 0.25). The serine concentration decreased by 15 to 18%, perhaps because of the elevation of phosphoserine (Table XXV). This decrease of essential amino acid concentrations and increase of non-essential amino acid concentrations shows an impairment of the nutritional status. That is, they have less essential amino acids available for protein synthesis.

There is a slight decrease (7%) of total essential amino acid and a small decrease (11%) of non-essential amino acids, in RD rats (Table XXVI). This is in good agreement with the normal BUN level and the very increased protein synthesis in RD rats (our data as described above) and is in consonance with the elevated activities of the urea cycle enzymes in uremic rats.

It has been reported that recycled urea nitrogen is used in uremia for the synthesis of non-essential amino acids (141-143). The nitrogen is also available for the synthesis of some essential amino acids if their carbon skeletons are provided in the diet (144-145); perhaps the nephrectomized rats could not use this alternative while RD rats could. It has been shown (146,147,155) that protein restriction alone reduced the activity of urea cycle enzymes - arginase synthetase system enzymes, alanine amino transferase (AIT) and branched chain aminotransaminase (BATase) but uremia alone increased these activities and particularly the activity of argininosuccinate synthetase; the rate limiting enzyme in the urea cycle is increased in uremia in direct proportion to the BUN concentration. Muting et al. (148) observed that concentrations of α-amino nitrogen particularly lysine, tyrosine, tryptophan, methionine, glycine, and glutamine were normal to 3 times greater in uremic serum. The non-uniformity of some amino acids may be due to the difference in their separate amino acid transport pathways and the difference in the affinities of amino acids for the particular transport site or mechanism (149).

In the nephrotic rats as well as RD rats the depressions of both dietary essential amino acids (valine, tryptophans) and non-essential amino acid (tyrosine) may indicate that depression of specific intracellular amino acids which can lead to impaired protein synthesis may exist in uremia (157).

These support our findings very well. The clinical implications of our findings are that if proper dietary care is not taken, dialysis, a common uremia treatment in human subjects may cause further depletion of some of these amino acids.

In our experiment the phenylalanine/tyrosine ratio (9.21/10.5 in U rats and 8.8/12.0 in RD rats) is not significantly increased, showing that the phenylalanine hydroxylase enzyme system is normal. This reflects the results of others (152).

Shear observed, in bilaterally nephrectomized rats, an increased uptake of essential amino acids by the liver, coupled with an increased rate of RNA and protein synthesis and a decrease in the plasma concentrations of essential amino acids (152). Our observations are in line with this.

(b) Glucogenic or Glycogenic and Ketogenic Amino Acids

In uremic rats total glucogenic amino acids levels increased by 6% while in RD rats they decreased by 9%, showing the depression of the gluconeogenic pathway in U and elevation in RD rats when compared to SO groups. These observations are supported by our data on SGOT (decreased in U and elevated in RD when compared to SO). The total ketogenic amino acid concentration in both U (by 14%) and RD rats (by 8%) decreases significantly. This also suggested that gluconeogenesis decreases while ketogenesis associated with lipogenesis increases in uremics. This correlates well with the earlier findings of G-6-PDH activity, and with protein and lipid data, to suggest lowered gluconeogenesis accompanied by increased glycolysis up to the G-6-P level and then increased oxidative pathway and decreased SGOT, lipogenesis in the nephrotic rats. Our findings are in agreement with most workers who found that in uremia several glucogenic amino acids (aspartate, glutamate, alanine) elevate and their corresponding metabolites of TCA cycle (malate, oxaloacetate and α-ketoglutarate) also elevate in nephrotic rat due to peripheral mobilization of amino acids and increased amino acid metabolic enzymes of the liver (135,136,150). However, valine being glycogenic and anti-ketogenic (402,403) shows a decrease of 11 to 24% in U rats and a 10% decrease in RD rats. This may support the glycogenesis we have seen in the nephrotic and RD rats, and should be considered individually.

(c) 1-Methyl and 3-Methyl Histidine, and Histidine

Histidine is an important biological compound. The compound carnosine (β-alanyl histidine) and anserine (β-alanyl-1-methyl histidine), derivatives of histidine, occur in muscle (407). Anserine was depleted by 22% in U and 7% in RD rats. The histidine and 1-methyl histidine profiles of uremic plasma are comparable to those of SO and

RD rats. Very surprisingly, 3-methyl histidine could not be detected in SO or RD plasma at all. This surely can be used to monitor the onset of uremia as well as progressive deterioration along with SGOT and BUN. 1-methyl histidine increases by 56% to 147% in U plasma and increases only by 10% in RD plasma compared to SO. However, the plasma aminogram obtained on dialysis by other workers (139,141, 143,145) have shown elevated 1-methyl and 3-methyl histidine and notably proline and hydroxyproline.

The rates of accumulation of 3-methyl histidine in body fluids of uremics, particularly anephric patients, are known to provide a method to estimate the rate of catabolism of muscle protein. The elevation of 1-methyl and 3-methyl histidine are said to be a consequence of failure of renal clearance. The compound 3-methyl histidine is of particular interest in view of the fact that this is uniquely found in the actin portion of the major muscle protein, actinomyosin and rate of its appearance might be a valuable index of muscle protein catabolism (405). Perhaps, we for the first time, showed the presence of 3-methyl histidine in nephrotic rat plasma and its absence from SO and RD plasma. In RD surprisingly, protein is presumably utilized yet no 3-methyl histidine could be detected.

(d) Serine, Phosphoserine, Taurine, Methionine

In uremic plasma phosphoserine increased by 65 to 266% ($P \leq 0.005$) and in contrast only by 12% in RD ($P \leq 0.45$; not significant), compared to SO plasma. This is another parameter which might be of use as a valuable index for monitoring the onset of uremia.

Phosphoserine is obtained by reversal of transamination of phosphohydroxy pyruvic acid and glutamate. Phosphohydroxypyruvic acid is formed from phosphoglyceric acid. Thus, glyceric or 3-phosphoglyceric acids are the precursors of phosphoserine (406). This then supports the elevated lipogenesis as we earlier reported.

Sulfur containing amino acids, taurine, cystine and methionine are of interest. Taurine was elevated by 7 to 19% ($P \leq .20$ to .35) (not significant) cystine by 13 to 52% ($P \leq 0.25$) and methionine by 12% (with a decrease of 9% in the first period; $P \leq 0.10$) in uremic rats. This statistically insignificant rise of metabolic acid forming amino acids support very well our findings of alkaline urinary pH (Table X) further in favour of total alkalosis favouring a decrease in gluconeogenesis as is often suggested (74).

(e) Proline and Hydroxyproline

Uremic plasma hydroxy proline was elevated by 30 to 43% ($P \leq 0.10$ to 0.01) and proline by 6 to 27% ($P \leq 0.20$

to 0.25; insignificant) where as both decreased in RD plasma, only hydroxyproline significantly compared to SO rat. The increase of hydroxyproline indicate bone disturbances as suggested by others (168). The elevation of proline and hydroxyproline is an index of collagen turnover in primary hyperparathyroidism and chronic uremia (154).

(f) Branched Chain Amino Acids (Valine, Isoleucine, Leucine, and Aromatic Amino Acids)

Of the branched chain amino acids leucine and valine are decreased in U plasma; all were unchanged in RD plasma. This contradicts the findings of Bertstrom _et_ _al_. (145,185).

Of the aromatic amino acids, the ratio of phenylalanine to tyrosine suggest no abnormality of phenylalanine hydroxylase enzyme.

Morgan _et_ _al_. (156) reported that aromatic amines elevate in uremia and that their elevation parallels BUN concentration, but fails to show quantitatively (157,158, 159).

(g) Urea-Cycle Amino Acids

Of the urea-cycle amino acids, ornithine increased by 9% (P≤0.15) in U plasma and decreased by 42% (P≤0.0005) in RD; aspartic increased by 30 to 36% in U plasma (P≤0.10 to 0.15) and decreased by 31% (P≤0.20) in

RD, and arginine decreased by 18 to 22 (0.15 to 0.0125) in U plasma and by 10% ($P \leq 0.40$) in RD plasma. This is what would be expected in view of BUN concentration and impact of urea cycle enzyme in U and RD.

(h) Unnatural Amino Acids
(α-Amino Adipic Acid and α-Amino n-Butyric Acid)

α-amino adipic acid increased by 41 to 121% ($P \leq 0.10$ to 0.0025) in U plasma and decreased by 10% ($P < 0.35$, insignificant) in RD plasma, whereas α-amino-n-butyric acid showed an insignificant decrease of 13 to 15% in U plasma and elevation of 23% ($P \leq 0.20$) in RD plasma. This inverse change of these two amino acids (in U compared to RD rats), which do not occur normally in protein (and hence are unnatural delineat the difference between the uremic effect and the starvation effect in these amino acids concentration in plasma.

α-amino butyric acid is found in natural materials - in non-protein extracts of animal tissue. Pre-cursors of this amino acid in the animal organisms are threonine (407) and methionine (408,409). Surprisingly enough, the decrease of threonine in our U rats was insignificant whereas in RD rats the decrease is sufficient, 19% ($P \leq 0.05$) to correlate with the change of the level of α-amino-butyric acid. α-amino butyric acid also appeared in urine of hepatic diseased patients following the administration of methionine, and enzymatically cleared product of threonine.

α-amino adipic acid, a catalytic product of lysine (410) showed significant increase in the U rats, while the lysine concentration showed no significant change. However, the insignificant decrease of α-amino adipic acid in RD plasma also does not correlate with lysine concentrations in RD rat plasma.

CHAPTER VI

SUMMARY

Sub-total nephrectomy in rats has been used to elicit a uremic condition in the animals. This uremia has been studied and progressively monitored by BUN.

BUN elevation was not always maintained in these animals; a compensatory decrease in BUN occurred in some cases, even when the overall uremic effect prevailed and the condition of the rats deteriorated. This prompted an investigation of SGOT, serum CPK, plasma alkaline phosphatase, hematocrit, plasma and urinary glucose and the aminogram of plasma. Control animals used were sham operated rats and restricted diet rats; the latter group served as controls for the starvation due to anorexia, to delineate vividly the effects of uremia from the secondary effects of starvation.

SGOT and plasma 3-methyl histidine are the two parameters which came to be outstanding criteria for monitoring the onset as well as the progressive deterioration of uremia in nephrectomized rats. The 50% decrease of SGOT over the effect of starvation allowed the detection of uremic effects even when BUN compensation occurred. 3-methyl histidine very clearly signaled kidney failure, as it can be seen only in uremic plasma.

The influence of sub-total nephrectomy on glucose metabolism was studied by estimating plasma glucose; the uremic rats were found to be hypoglycemic. The plausible causes of hypoglycemia were investigated by estimating urinary glucose, hepatic glycogen content, hepatic glycogen structure, *in vivo* oxidation of ^{14}C-glucose to $^{14}CO_2$, and its *in vivo* conversion to liver and plasma protein, liver glycogen, muscle, liver and adipose tissue lipid and plasma protein and non-protein and free fatty acids.

The abnormal deposition of glycogen in the uremic liver and the abnormal uremic hepatic glycogen structure were found to be the cause of hypoglycemia, as no glucosuria was present. The changes in intra-molecular structure of the uremic hepatic glycogen were:

i. an increased number of branching points (i.e. 1,6-bonds),

ii. an increased number of segments per mole,

iii. a decreased number of glucose residues per segment and

iv. an increased number of non-reducing ends.

This abnormal glycogen structure in the uremic liver correlated well with the measured activities of the glycogen cycle enzymes. Amylo-1,6-glucosidase activity showed an inverse relationship with the activity of glycogen synthetase and the α-glucan phosphorylase ratio (of ("a" to "a" +

"b") was doubled. This indicates that the control mechanism is such that when the chain length increase occurs it is counteracted by reducing synthetase activity and by reducing the activity of amylo-glucosidase; again, when branching points start increasing, it is controlled by reducing the activity of synthetase and increasing the activity of amylo-1,6-glucosidase. In the uremic environment the fine control mechanism shows abnormal function. In other words, when the ATP and ATP generating system is available, glucose is converted to glycogen via amylo-1,6-glucosidase; at other times, when G-6-P or UDPG is available then there is conversion to glycogen caused by glycogen synthetase. At another time, when [P_i] and [G-1-P] are favourable, the formation of glycogen occurs by α-glucan phosphorylase. It also shows that as long as glucose is available amylo-1,6-glucosidase is active.

The excessive conversion of ^{14}C-glucose to $^{14}CO_2$ and the increased activity of G-6-PDH in uremia, coupled with a decrease of SGOT, an increase of plasma glucogenic amino acids, an alkaline urinary pH, and a normal concentration of sulfur containing amino acids (cystine, taurine, methionine) confirm that there is increased glycolysis up to the level of G-6-P; this is perhaps due to the Pasteur effect (inhibition of anaerobic glycolysis), shifting to the pentose monophosphate shunt and decreasing gluconeogenesis. In addition to this, studies of the in vivo conversion of ^{14}C-glucose into lipid, protein and glycogen revealed increased specific activities of lipid in muscle, liver and adipose tissue, of protein in liver and plasma and of glycogen in liver. This suggests lipogenesis and mobilization of the reserve of peripheral tissue lipid, carbohydrate and protein. Thus sub-total nephrectomy in rats was found to cause:

1. Hypoglycemia
2. Abnormal deposition of liver glycogen
3. Changes in the intra-molecular structure of hepatic glycogen
4. Derangement of glycogen cycle enzyme activities
5. Increased activity of the oxidative pathway and an increased rate of glycolysis above glucose-6-P, associated with lipogenesis
6. Decreased gluconeogenesis
7. Decreased SGOT
8. The appearance of plasma 3-methyl histidine and the elevation of plasma phosphoserine and 1-methyl histidine

A shift in the pattern of glucose utilization by preferentially channeling a larger portion of glucose for production of tissue lipid and protein from the peripheral lipid reserves.

The Summary is outlined in Fig. 35, showing the pathways studied in the nephrotic rat.

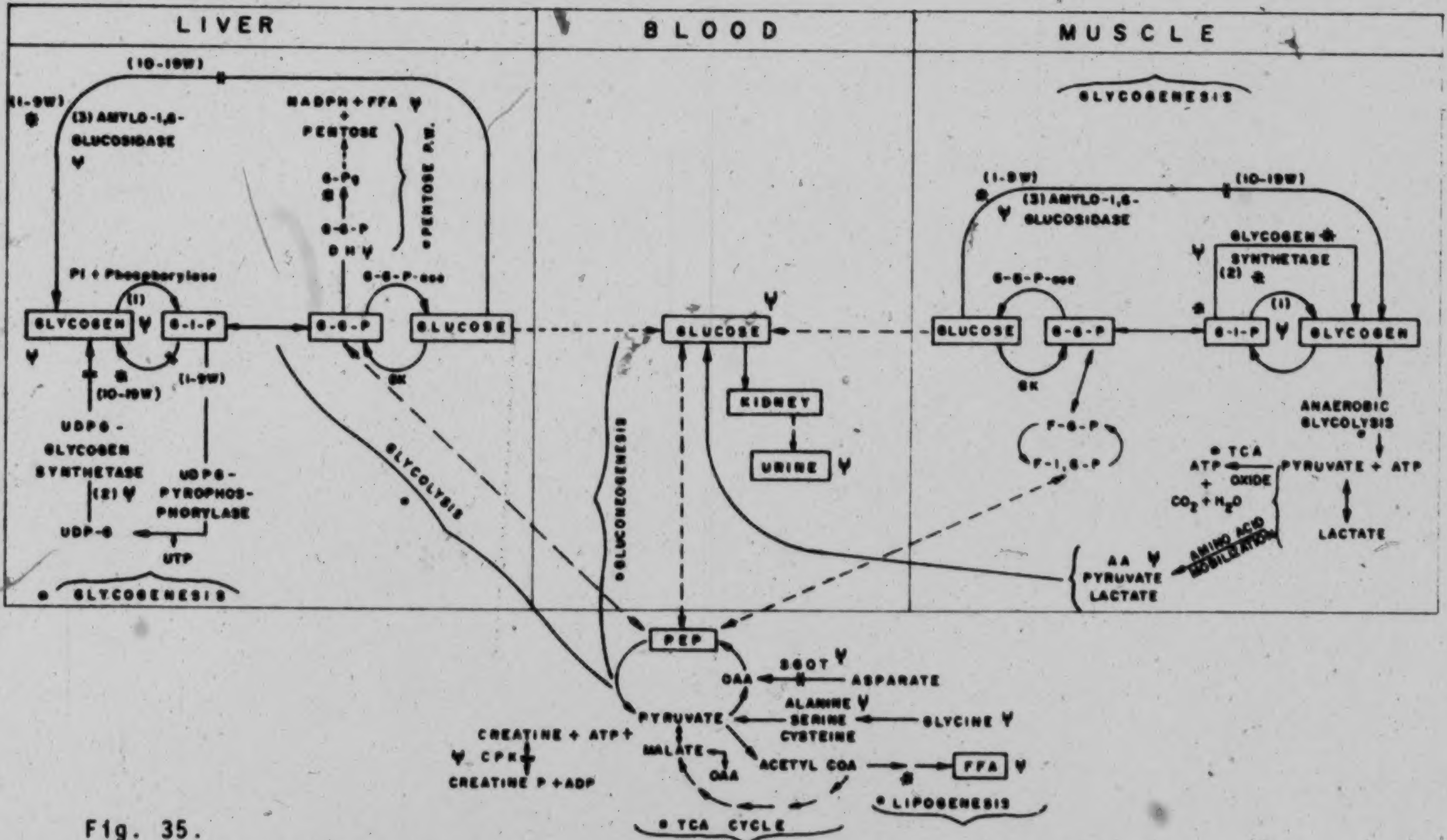

LIVER
BLOOD
MUSCLE
(10-19W)
(1-9W)
(3) AMYLO-1,6-GLUCOSIDASE
NADPH + FFA
PENTOSE
6-Pg
6-G-P
D H
PENTOSE P.W.
Pi + Phosphorylase
(1)
GLYCOGEN
G-1-P
G-6-P
G-6-P-ase
GLUCOSE
GK
(10-19W)
(1-9W)
UDPG-GLYCOGEN SYNTHETASE
(2)
UDPG-PYROPHOSPHORYLASE
UDP-G
UTP
GLYCOGENESIS
GLYCOLYSIS
GLUCONEOGENESIS
GLUCOSE
KIDNEY
URINE
GLYCOGENESIS
(1-9W)
(10-19W)
(3) AMYLO-1,6-GLUCOSIDASE
GLYCOGEN SYNTHETASE
(2)
G-6-P-ase
GLUCOSE
G-6-P
G-1-P
(1)
GLYCOGEN
GK
F-6-P
F-1,6-P
ANAEROBIC GLYCOLYSIS
TCA
ATP
OXIDE
CO₂ + H₂O
PYRUVATE + ATP
LACTATE
AMINO ACID MOBILIZATION
AA
PYRUVATE
LACTATE
PEP
OAA
SGOT
ASPARATE
ALANINE
SERINE
CYSTEINE
GLYCINE
PYRUVATE
CREATINE + ATP
CPK
CREATINE P + ADP
MALATE
OAA
ACETYL COA
FFA
LIPOGENESIS
TCA CYCLE

Fig. 35.

BIOCHEMICAL PARAMETERS STUDIED IN NEPHERECTOMIZED RATS

Ψ POINTS OF STUDY MADE; # INHIBITION; ✱ ACTIVATION; • CYCLES IN OPERATION;
(1), (2), (3) INDICATE MAIN PATHWAYS IN GLYCOGENESIS

CHAPTER VII

REFERENCES

1. The Biochemical Consequence of Chronic Renal Failure, Wills, M.R., Harvey Miller & Medcalf (1971).

2. Hicks, J.M., Young, D.S., and Wootton, I.D.P., (1964), Clinica Chim. Avta., 9, 228.

3. Rosenheim, M.L., and Ross, E.J., (1967): Chronic Renal Failure in Renal Disease, 2nd Ed., 327-349, Edit: D.K. Black, Pub. Blackwell Scientific Publications, Oxford.

4. Berliner, R.W.; Outline of Renal Physiology. In Strauss and Welt, Eds. Diseases of the Kidney, Boston, Little, Brown & Co. (1963).

5. Pilts, R.F.; Physiology of Kidney and Body Fluids, Chicago, Yearbook Medical Publishers, Inc. (1963).

6. Smith, H.W., (1956), Principles of Renal Physiology, Pub. Oxford University, Press Inc., N.Y.

7. Mattenheimer, H., In Clinical Enzymology: Principles and Applications, Ann Arbor Sci. Publishers Inc. (1971).

8. Enzymology of Kidney Tissues: In Enzymes in Urine and Kideny, Ed. U.C. Dubach, (1968), Hans Huber Verlag.

9. Pollak, V.E., et al., (1965), J. Clin. Invest., 44, 169.

10. Gault, M.H., and Giggi, P.H.S., (1970), Canada Med. Ass. J., 101, 208.

11. Amador, E., (1970), Postgrad. Med., 47, (3), 59.

12. Kemp, E., Lange, H., Laurson, T.E., Nielson, V.K., (1964), Proc. Europ. Dial, Transpi. Ass. 1, 135.

13. Ringoir, S., Wiemer, R., and Regniers, P., (1965), Proc. Europ. Dial. Transpl. Ass., 2, 191.

14. Escher, J., and Zimmerman, H.J., (1967), Am. J. Med. Sci., 253, 272.

15. Sharratt, M., and Frazer, A.C., (1963), Toxic Appl. Pharmac., 5, 36.

16. Brown, H., Saleeby, E.R., and Shamberg, J.F., (1926), J. Pharmac. Exp. Ther., 28, 141.

17. Fennel, R.H., and Pardo, V.M., (1967), Lab. Invest., 17, 481.

18. Berg, B.N., (1966), Proc. Soc. Biol. Med., 121, 198.

19. Street, A.E., Chesterman, H.; Smith, S.K.A., and Quinton, R.M., (1968), Toxic. Appl. Pharmac., 13, 363.

20. Schriener, G.E., and Maher, J.F.: Uremia: Biochemistry, Pathogenesis and Treatment, Springfield, Thomas, (1961), 55-55, 94.

21. Wills, M.R., (1968), Biochemical Consequences of Chronic Renal Failure, A Review, J. Clin. Path., 21, 541.

22. Karl, I.E., Garcia, P., White, W.L., Recant, L., and Kissane, J.M., (1964), Lab. Invest., 13, 1600.

23. Bealty, L.D., Dodson, V.N.; and Cornish, H.H., (1966), Toxic. Appl. Pharmac., 7, 480.

24. Gault, M.H., and Giggie, P.H.S., (1969), Can. Med. Ass. J., 101, 208.

25. Amador, E., Dorfman, L.E., and Wacker, W.E.C., (1965), Ann. Intern. Med., 62, 30.

26. Bank, N., and Bailine, S.H., (1965), New Eng. J. Med., 272, 70.

27. Noble, R.E., Najarian, J.S., and Brainard, H.D., (1965), Proc. Soc. Exp. Biol. Med., 120, 737.

28. Harrison, J.F., et al., (1968), Lancet., 1, 371.

29. Braudi, A.I., and Berkowitz, H., (1961), J. Lab. Clin. Med., 57, 490.

30. Schoenfeld, M.R., (1965), J. A.M.A., 193, 618.

31. Mason, E.E., and Dierks, C., (1961), Bull. Soc. Int. Chir., 20, 173.

32. Dunn, M., Martins, J., and Reissmann, K.R., (1959), J. Lab. Clin. Med., 51, 254.

33. Chinsky, M., Shmagranoff, G.L., and Sherry, S., (1956), J. Lab. Clin. Med., 47, 108.

34. Rowntree, L.G., and Geraghty, J.T., (1910), J. Pharm. and Exp. Therap., 1, 579.

35. Smith, H.W., (1956), Principles of Renal Physiology, New York, Oxford University Press Inc.

36. Sharratt, M., (1958), The Effect of Food on Renal Function,; Thesis, University of Birmingham, England.

37. Moller, E., McIntosh, J.F., and Van Shyke, D.D., (1929), J. Clin. Invest., 6, 427.

38. Jolliffe, N., and Smith, H.W., (1931), Am. J. Physiol., 98, 572.

39. Smith, H.W., (1951), The Kidney, Structure and Function in Health and Disease, New York, Oxford University Press Inc.

40. Murphy, G.P., Mirand, E.A., Johnston, G.S., Schmidt, J.D., and Scott, W.W., (1966), Investve. Urol. 4, 39.

41. Blaufox, M.D., and Merril, J.P., (1956), Fed. Proc., 24, 405.

42. Barger, A.C., (1966), Ann. N.Y., Acad. Sci., 139, 276.

43. Rosenthall, I., and Ried, E.C.: Radionuclide Distinction of Vascular and Non-vascular Lesions of the Kidney, (1968), Canada Med. Ass. J., 98, 1165.

44. Personal Communication, Prof. D.S. Silverberg, Faculty of Medicine, University of Alberta, (Hospital: Uremics Ward) (1973).

45. Kouglas, A.P., and Kerr, D.N.S., A Short Textbook of Kidney Disease, J.P. Lippincott Co., Philadelphia, (1960); Krupp, M.A., and et al., Current Diagnosis and Treatment, Lange Medical Publication, Los Angeles, California (1971).

46. Peters, J.P., (1935), Body Water, Publisher, Balliere, Tindall and Cox, London.

47. Grollman, E.F., and Grollman, A., (1959), J. Clin. Invest., 33, 749.

48. Foster, N.B., (1915), Trans. Ass. Am. Physns., 30, 305.

49. Harrison, T.R., and Mason, M.F., (1937), The Pathogenesis of the Uramic Syndrome, Medicine, Baltimore, 16, 1-44.

50. Olsen, N.S., and Bassett, J.W., (1951), BUN, Phenol, Guanidine and Creatinine in Uremic, Am. J. Med., 10, 52.

51. Giovannetti, S., Biagini, M., Balestri, P.L., Navalisi, R., Giagnoni, P., De Matteis, A., Ferri-Milone, P., and Perfetti, C., IVth Int. Cong Nephrol, Stockholm, June 22-27 (1969), 43.

52. Seminhoff, M.L., Asatoor, A.M., Milner, M.D., and Zilva, J.F., (1963), Clin. Sci., 25, 65-77.

53. Kramer, B., Seligson, H., Baltrush, H., and Seligson, D., (1965), Clinica Chim. Act., 11, 363-371.

54. Muting, D., (1965), Clinica Chim. Acta., 12, 551.

55. Bonas, J.E., Cohen, B.D., and Natelson, S., (1963), Michrochem. J., 7, 63.

56. Natelson, S., Stein, F., and Bonas, J.E., (1964), Michro. Chem. J., 8, 371.

57. Cohen, B.D., Stein, I.M., and Bonas, J.E., (1968), Am. J. Med., 45, 63.

58. Stein, I.M., Cohen, B.D., and Kornhauser, R.S., (1969), New England, J. Med., 280, 926.

59. Giovanetti, S., Biagini, M., and Cioni, L., (1968), Experientia, 24, 341, Giovanetti, S., Cioni, L., Balestri, P.L., and Biagini, M., (1968), Clin. Sci., 34, 141.

60. Giovanetti, S., Giagini, M., Balestri, P.L., Navalese, R., Giagnoni, P., De Matteis, A., Ferro-Milone, P., and Perfelti, C., (1969), Clin. Sci., 36, 445-452.

61. Editorial: A Protein Free Diet for Chronic Renal Failure, (1967), Lancet, 2, 874.

62. Krebs, H.A., and Hinseleit, H., (1932), Z. Physiol. Chem., 210, 33.

63. Grisolia, S., Phosphoru. Metabolism, Vol. 1, 619, (1951), Ratner's, ibid, 601.

64. Cohen, P.P., In Comparative Biochemistry, Vol. II, Florin, M., and Mason, H.S., (Eds), pp. 161-231, Academic Press, N.Y., (1960), In Chemical Pathways of Metabolism, Vol. II, Grenneberg, D.M., (Ed), pp. 1-46, (Academic Press, N.Y.), (1954), ibid, Cohen, P.P.

65. Hughes, D.E., (1950), Biochem. J., 46, 231, Brownstein, A.E., and Azarkh, R.M., (1939), BioKhimiya, 5, 1.

66. Cohen, B.D., and Horowitz, H.I., (1968), Am. J. Clin. Nutr., 21, 407.

67. Renal Failure, (Ed), Brest, A.N., and Meyer, J.H., J.B. Co. Lippincott (1967).

68. Erslev, A., The Kidney, Vol. I, No. 4, (1968), The Anemia of Renal Failure.

69. Relman, A.S., Adv. Int. Med., XII, 295 (1964).

70. Pitts, R.F., and Alexander, R.S., Nature of the Renal Tubular Mechanism for Acidifying the Urine, Am. J. Physiol., 144, 239 (1945).

71. Struyvenberg, A., Morrison, R.B.I., and Relman, A.S., (1966), J. Clin. Invest., 45, 1077.

72. Pellegrino, E.D., and Blitz, R.M., (1965), Medicine, 44, 397.

73. Kaye, M., (1964), J. Clin. Invest., 43, 1367.

74. Lemann, J., Jr., Goodman, A.D., Lennon, E.J., and Relman, A.S., (1963), J. Clin. Invest., 43, 951, Lemann et al., (1959): ibid, 38, 2215.

75. Dorhout, Mees, E.J., Machado, M., Slatopolsky, E., Klahr, S., and Bricker, N.S., (1966), J. Clin. Invest., 45, 289.

76. Relman, A.S., (1968), Am. J. Med., 44, 706.

77. Schwartz, W.B., and Relman, A.S., (1957), New England, J. Med., 256, 1184.

78. Wasserman, R.H., and Taylor, A.N., (1966), Science, N.Y., 152, 791; Wasserman, R.H., (1968), Calcified Tissue Res., 2, 301.

79. Deluca, H.F., (1969), Am. J. Clin. Nutr., 22, 412, ibid, (1970), Arch. Intern. Med., 126, 896.

80. Nordin, B.E.C., and Peacock, M., (1969), Lancet, 2, 1280.

81. Irving, J.T., (1964), Dynamics and Functions of Phosphorus in Mineral Metabolism, an Adv. Treatise, Vol. 2, Pt. A., p. 249, (Edit) C-L, Comar and F.C. Bronner, Pub. Academic Press Inc., (London) Ltd., London.

82. Walser, M., (1960), J. Clin. Invest., 39, 506.
Walser, M., (1961), J. Clin. Invest., 40, 723.

83. Fourman, P., and Morgan, D.B., (1969), Effect of Vit. D. in the Human, in Nutritional Aspects of the Devel. of Bone and Commective Tissues, p. 30-43, (Edit) Somoggi, J.C., and Kodicek, E., Bibliotheca Nutritio et Dieta, No. 13, Pub. Karger, Basel.

84. Fourman, I.P., and Reyer, P., (1968), Calcium Metabolism and the Bone, 2nd Ed., Pub. Blackwell Scientific Publications, Oxford.

85. Halver, B., (1966), Acta. Med. Sand., 179, 427.

86. Transbol, I., and Halver, B., (1967), J. Clin. Endocr. Metals, 27, 1193.

87. Morrin, P.A.F., Joynt, M.S.K., and Frame, J., (1970), Nephron, 7, 248.

88. Norman, A.W., Haussler, M.R., Adams, T.H., Mertle, J.F., Roberts, P., and Hubberd, K.A., (1969), Am. J. Clin. Nutr., 22, 396.

89. Avioli, L.V., Birge, S., Lee, S.W., and Slatopolsky, E., (1968), J. Clin. Invest., 47, 2239, Avioli, L.V., Lee, S.W., Birge, S.J., Slatopolsky, E., and De Luca, H.F., (1969), J. Clin. Invest., 48, 4a; Avioli, L.V., Scott, S., Lee, S.W., and De Luca, H.F., (1969), Science, N.Y., 166, 1154.

90. Chan, L.R., Fedak, S.A., and Aurbach, G.D., (1969), Endocrinology, 84, 761.

91. Vae, G., (1968), Proc. Third Parathyroid Conf., Montreal, (1967), (Edit) Talmage, R.V., and Belanger, L.F., Pub. Excerpta Medica Foundation, Excerpta Medica International Congress Series No. 159.

92. Murad, F., Brewer, H.B. Jo., and Vaughan, M., (1970), Proc. Natn. Acad. Sci. U.S.A., 65, 446.

93. Au, W.Y.W., and Raisz, L.G., (1967), J. Clin. Invest., 46, 1572.

94. Raisz, L.G., and Nieman, I., (1969), Endocrinology, 85, 446.

95. Butcher, R.W., Robinson, G.A., Hardman, J.G., and Sutherland, E.W., (1968), The Role of Cyclic AMP in Hormone Actions; In Adv. in Enz. Regulations, Vol. 6, p. 357, (Edit) G. Weber, Pub. Pergamon Press, Oxford.

96. Coleman, A.J., Arias, M., Carter, N.W., Rector, F.C.Jr., and Seldin, D.W., (1966), J. Clin. Invest., 45, 1116.

97. Cope, C.L., and Pearson, J., (1963), Clin. Sci., 25, 331.

98. De Wardener, H.E., (1962), In Renal Disease, (Edit) D.A.K. Black, Blackwell, Oxford.

99. Milne, M.D., (1963): In Disease of Kidney, (Edit) M.B. Strauss, and L.G. Welt, Little Brown, Boston.

100. Englert, E.Jr., Brown, H., Willardson, D.G., Wallach, S., and Simons, E.L., (1958), J. Clin. Endocr., 18, 36.

101. Shaldon, S., (1966), Postgrad. Med. J., 42, November Suppl.

102. Shaldon, S., Bailod, R., Comty, C., Oakly, J., and Switt, L., (1964), Proc. Europ. Dial. Transpl. Ass., 1, 233.

103. Salvesen, H.A., and Linder, G.C., (1923), J. Biol. Chem., 58, 617.

104. Merril, J.P., (1965), In the Treatment of Renal Failures, Grune and Stralton, N.Y.

105. Brun, M., and Marshall, R.T., (1966), J. Lab. Clin. Med., 68, 701.

106. Hamburger, J., (1957), Clin. Chem., 3, 332.

107. Randall, R.E.Jr., Cohen, M.D., Spray, C.C.Jr., and Losemeisl, E.C., (1964), Ann. Intern. Med., 61, 73.

108. Clarkson, E.M., McDonald, S.J., De Wardener, H.E., and Warren, R., (1965), Clin. Sci., 28, 107.

109. Clarkson, E.M., Warren, R.L., McDonald, S.J., and De Wardener, H.E., (1967), Clin. Sci., 32, 11.

110. Joske, R.A., McAlister, J.M., and Prankerd, T.A.Jr., (1956), Clin. Sci., 15, 511.

111. Loge, J.P., Lange, R.D., and Moore, C.V., (1958), Amer. J. Med., 24, 4.

112. Reissman, K.R., (1950), Blood, 5, 372.

113. Jacobson, L.O., Goldwasser, E., Fried, W., and Pizak, L., (1957), Nature (Lond), 179, 633.

114. Goldwasser, E., Fried, W., and Jacobson, L.O., (1958), J. Lab. Clin. Med., 52, 375.

115. Naets, J.P., (1960), Blood, 16, 1770; (1960), J. Clin. Invest., 39, 102.

116. Naets, J.P., and Heuse, A.F., (1962), J. Lab. Clin. Med., 60, 365.

117. Gallagher, N.I., McCorthy, J.M., Hart, K.T., and Lange, R.D., (1969), Blood, 14, 662.

118. Suki, W., and Grollman, A., (1960), Amer. J. Physiol., 199, 629.

119. Shaw, A.B., (1967), Brit. Med. J., 2, 213.

120. Castaldi, P.A., Rozenberg, M.C., and Stewart, J.H., (1966), Lancet, 2, 66.

121. Steward, J.H., and Castaldi, P.A., (1967), Quart. J. Med., 26, 409.

122. Mathews, D.M., and Beckett, A.G., (1962), J. Clin. Path., 15, 456.

123. Lasker, N., Harvey, A., and Baker, H., (1963), Trans. Amer. Soc. Artif. Intern. Org., 9, 51.

124. Shaldon, S., (1966), Postgrad. Med. J., 42, Nov. Suppl., Erslev, A.G., (1960), Control of Red Cell Productions, Ann. Rev. Med., 11, 315.

125. Reissman, K.R., Nomura, T., Gunn, R.W., and Brosius, F., (1960), Blood, 16, 1411.

126. Erslev, A.J., (1961), Trans. N.Y. Acad. Sci., 24, 13.

127. Lacy, W.W., (1965), Clin. Res., 13, 80.

128. McCormick, G.J., Shear, L., and Barry, K.G., (1966), Proc. Soc. Exp. Biol., (N.Y.), 122, 99.

129. Herdon, R.F., Freeman, S., and Cleveland, A.S., (1958), J. Lab. Clin. Med., 52, 235.

130. Hume, D.M., Merril, J.P., Miller, B.F., and Thorn, G.W., (1955), J. Clin. Invest., 34, 327.

131. Dammin, G.J., Couch, N.P., and Murray, J.E., (1957), Ann. N.Y., Acad. Sci., 64, 967.

132. Smiddy, F.G., Burwell, R.G., and Parsons, F.M., (1961), Nature (London), 190, 732.

133. Kirkpatrick, C.H., Wilson, W.E.C., and Talmage, D.W., (1964), J. Exp. Med., 119, 727.

134. Rowland, D.T.Jr., Wilson, W.E.C., and Kirkpatrick, C.H (1964), J. Allergy, 35, 242.

135. Bennington, J.L., Haber, S.L., Smith, J.V., and Warner, N.E., (1964), Am. J. Clin. Path., 41, 8.

136. Samec, M., and Isajevic, V., (1923), Compt Renal, 176, 1419.

137. Mathews, D.M., Laster, L., (1965), A Review; Gut 6, 411.

138. Gulyassy, P.F., Aviram, A., Peters, J.H., (1970), Arch Intern Med., 126, 855 (1970).

139. Giordano, C., dePascale, C., de Santo, N.G., Furst, P., Stangherlin, P., (1970), Arch Intern. Med., 126, 787.

140. Walser, M., Bodenlos, L., (1969), J. Clin. Invest., 38, 1617.

141. Giordano, C., (1963), J. Lab. Clin. Med., 62, 231.

142. Richards, P., Metcalfe, G.A., Ward, E.F., Wrong, M., Houghton, B.J., (1967), Lancet ii, 845.

143. Giordano, C., de Pascale, C., Balestris, C., Ciltadine, D., Crescenzi, A., (1968), Am. J. Clin. Nutri., 21, 394.

144. Richards, P., Brown, C.L., Houghton, B.J., Thompson, E.E., (1971), Lancet ii, 128.

145. Giordano, C., Phillips, M.E., dePascali, C., de Santo, N.G., Furst, P., Brown, C.L., Houghton, B.J., Richards, P., (1972), Lancet i, 178.

146. Brown, C.L., Houghton, B.J., Souhami, R.L., and Richards, P., (1972), Clin. Sci., 43, 371.

147. Walser, M., and Bodenlos, L.J., (1959), J. Clin. Invest., 38, 1617.

148. Muting, D., Dishuk, B.D., (1967), Proc. Expt. Biol. Med., 126, 754.

149. Christensen, H.N., (1962), Biological Transport. N.Y., W.A. Benjamen Inc. (1962).

150. Mayer, M., and Shafrir, E., Isr. J. Med. Sci., 8, 271, May (1972).

151. McGale, E.H.F., Pickford, J.C., Aber, G.M., (1972), Clin. Chim, Acta., 38, 395.

152. Shear, L., (1969), J. Clin. Invest., 48, 1252.

153. Loeper, M., Coltel, J., Lesure, A., Thomas, A., (1938), Compt. Renal. Soc. Biol, 128, 754.

154. Dubovsky, Y., Pacovsky, V., Dubovska, E., (1965), Clin. Chim. Acta., 12, 230.

155. Maier, K.P., Hoppe-Seyler, G., Talke, H., Frohlich, J., Scholmeyer, P., and Gerok, W., (1973), Europ. J. Clin. Invest., 3, 201.

156. Morgan, R.E., and Morgen, J.M., (1966), Metabolism 15, 479.

157. Gulassay, P.F., Peters, J.H., Lin, S.C., and Ryan, P.M., (1968), Am. J. Clin. Nutr., 21, 565.

158. Czerniak, Z., and Burzynski, S., (1969), Clinica. Chim. Acta., 24, 367.

159. Burzynski, S., (1969), Clinica. Chim. Acta., 25, 231.

160. Ludewig, S., and Chanutin, A., (1938), Arch. Intern. Med., 61, 847.

161. Bloomfield, A.L., (1933), J. Exp. Med., 57, 705.

162. Kark, R.M., Pirani, C.L., Pollock, V., Muehrceke, R.C., and Blainy, J.D., (1958), Ann Intern. Med., 49, 751.

163. Cameron, J.S., and Blanford, G., (1966), Lancet, 2, 242.

164. Randolph, M.F., and Greenfield, M., (1967), Amer. J. Dis. Child., 114, 613.

165. Levitt, J.I., (1968), Ann Intern. Med., 66, 685.

166. Peters, J.H., Gulyassey, P.F., Lin, S.C., Ryan, P.M., Berridge, B.J.Jr., Chao, W.R., and Cummings, J.G., (1968), Trans. Amer. Soc. Artif. Intern. Organs., 14, 405.

167. Josephson, B., Amino acids and Uremia. Abstracts General Sessions - Symposia, IVth Int. Cong. Nephrol., Stockholm, (1969), p. 134.

168. Kaye, J.P., Moorhead, J.F., and Will, M.R., (1973), Clinca. Chimica. Acta., 44, 301.

169. Teschan, P.E., (1970), Am. J. Med., 48, (6), 671.

170. Giordano, G., Bloom, J., and Merrill, J.P., (1962), J. Lab. Clin. Med., 59, 396.

171. Schmidt, E.G., McElvain, N.F., and Bowen, J.J., (1950), Am. J. Clin. Path., 20, 253.

172. Young, D.S., and Wootton, I.D.P., (1964), Clinica. Chim. Acta., 9, 503.

173. Schwerin, P., Bessman, S.P., and Waelsch, H., (1950), J. Biol. Chem., 184, 37.

174. Tholen, V.H., Bigier, F., (1964), Schweiz Med. Wschr., 94, 65.

175. Bock, H.E., Walter, H.D., Kaufmann, W., Lohr, G.W., (1965), Dtsch. Arch. Clin. Med., 211, 73.

176. Shafrir, E., Brener, T., Gutman, A., Orivi, M., Diamant, S., and Mayer, M., (1972), Isr. J. Med. Sci., 8, (1), 97.

177. Lonergan, E.T., Semar, M., Lange, K., (1970), Arch. Intern. Med., 136, 851.

178. Rajagopalan, K.V., Fredovich, I., Handler, P (1961), J. Biol. Chem., 236, 1059.

179. Cummings, N.B., Stetten, D.E.W.Jr., (Abs.), (1963), J. Clin. Invest., 42, 927.

180. Morgan, J.M., Morgan, R.E., Thomas, G.E., (1963), Metabolism, 12, 1051.

181. Clayton, E.M., Seligson, D., Seligson, H., (1965), Yale J. Biol. Med., 38, 273.

182. Glaze, R.P., Morgan, J.M., Morgan, R.E., (1967), Proc. Soc. Exp. Biol. Med., 125, 172.

183. Horowitz, H.I., Cohen, B.D., Martinniz, P., (1967), Blood, 30, 331.

184. Symposium: Nutritional Aspects of Uremia, Sessions III and IV. Amer. J. Clin. Nutr., 21, 547 and 407 (1968).

185. Renner, D.; Inter. Congress of Nephrology, Mexico, Oct. 8-13, (1972), Abstracts; Dzurik, R., Cernacek, P., Valovicora, E., Hupkove, V., Abstracts; Yamoda, T., Yoshida, A., Hatona, M., Abstracts; Duffy, *et al*., Abs. #984.

186. Liber, H.W., and Schrutterle, (1972), Kidney Internal 2, 152.

187. Blackall, J., (1813), Observations on the Nature and cure of Dropsis and Particularly on a Persence of the Coagulable Part of the Blood in Dropsical Urine, Pub. Longman, Hurst, Rees, Orme and Brown, London.

188. Christisen, R., (1839), On the Granular Degeneration of the Kidneys and its Connexion with Dropsy, Inflammations and Other Diseases, Pub. Adam and Charles Black, Edinburgh.

189. Roodvoets, A.P., VanNeerbos, B.R., Hooghwinkel, G.J.M., Hulsmans, H.A.M., and Beukers, (1967), Proc. Europ. Dial. Transpl. Ass., 4, 257-263.

190. Losowsky, M.S., and Kenward, D.H., (1968), J. Lab. Clin., Med., 71, 736.

191. Bagdade, J.D., Porte, D.Jr., and Bierman, E.L., (1968), New Engl. J. Med., 279, 181.

192. Bagdade, J.D., (1968), Am. J. Clin. Nutr., 21, 426.

193. Reimold, W.V., Kalterman, R., and Quellhorst, E., (1969), Arch. Klin. Med., 216, 384.

194. Heyman, W., (1962), Science, 96, 163.

195. Svanborg, A., (1951), Acta. Med. Scand. Supp., 264.

196. Winkler, A.W., Durlacher, S.H., Hoff, H.E., and Man, E.B., (1943), J. Exp. Med., 77, 473.

197. Lewis, L.A., Page, I.H., and Kolff, W.J., (1958), Amer. J., Physiol., 195, 161.

198. Jiminez-Diaz, C., and Castro-Mendoza, H., (1952), Rev. Clin. Esp., 44, 228.

199. *ibid*., (1948), Rev. Clin. Esp., 29, 84.

200. Lewis, L.A., Zuehlke, V., Nakamoto, S., Kolff, W.J., and Page, I.H., (1966), New Eng. J. Med., 275, 1097.

201. Fredrickson, D.S., Levy, R.I., and Lees, R., (1967), New Engl. J. Med., 276, 32-44, 94-103, 148-156, 215-221, 273-281.

202. Cohen, A.M., and Teitelbaum, A., (1964), Am. J. Physiol., 206, 105.

203. Ibid., (1966), Metabolism, 15, 1034.

204. Tzur, R., Tal, E., and Shapiro, B., (1964), Bio. Chem. Biophys. Acta., 84, 19.

205. Baxter, S.H., Goodman, H.C., and Shafrir, E., (1959), J. Clin. Invest., 38, 986.

206. McKenzie, I.F.C., and Nestel, P.S., (1968), J. Clin. Invest., 47, 1685.

207. Kerki, M., and Nikkila, E.A., (1971), Eur. J. Clin., Invest., 1, 345.

208. Marsh, J.B., and Whereat, A.F., (1959), J. Biol. Chem., 234, 3196.

209. Radding, C.M., and Steinberg, D., (1960), J. Clin. Invest., 39, 1560.

210. Wood, H.G., and Ulter, M.F., This Role of CO$_2$ Fixation in Metabolism, in P.N. Campbell and G.D., Grenovitch, (Eds.) "Essays in Biochemistry", Vol. I, pp. 1-28, Academic Press, Inc., N.Y., (1965), Ulten, M.F., (1958), Ann Rev. Biochem., 27, 245.

211. Krebs, H.A., and Kornberg, H.L., Energy Transformation in living matter. A survey, Springer, Berlin (1957).

212. Wood, W.A., Carbohydrate Metabolism, (1966), Ann. Rev. Biochem., 35, 485-520.

213. White, A., Handler, P., and Smith, E.L., In Principles of Biochemistry, pp. 406-411, McGraw-Hill Book Co., Toronto (1968).

214. West, E.S., and Todd, W.R., In Biochemistry, pp. 966-985, The MacMillan Co., N.Y. (1962).

215. Ashmore, J., and Weber, G., (1959), Vitamins and Hormones, 17, 91.

216. Hiatt, H.H., Goldstein, M., Lareau, J., and Horecker, B.L., (1958), J. Biol. Chem., 231, 303.

217. Wittstatter, R.A., Rohdewald, M., (1934), Hoppe-Seyler's X. Physical Chem., 225, 103, Mystkowski, E.M., (1935), Biochem., 278, 240.

218. Meyer, F., and Zalta, J.P., (1958), Compt. Rend. Acad., Sc., 247, 357.

219. Meyer, F., and Lourau, M., (1956), Compt. Rend. Acad. Sc., 242, 2184.

220. Schwimmer, S., (1950), J. Biol. Chem., 186, 181. Hanrahan, V.M., and Caldwell, M.L., (1953), J. Am. Chem. Soc., 75, 2191.

221. Myrback, K., Advances in Carbohydrate Chem., 3, 251, (1948).

222. Manners, D.J., Ann. Reots on Progr. Chem., (Chem. Soc. London), 50, 288, (1954), Quart. Revs. (London), 9, 73 (1955).

223. Illingworth, B., Larner, J., and Cori, G.T., (1952), J. Biol. Chem., 199, 631.

224. Whelan, W.J., (1953), Biochem. Soc. Symposia (Cambridge, Engl.), 11, 17.

225. Bobbit, J.M., Adv. in Carb. Chem., 11, 1 (1965).

226. Soskin, S., and Levine, R., Carbohydrate Metabolism, University of Chicago Press, Chicago, (1946), p. 12.

227. Mirski, A., Rosenbaum, I., Stein, L., and Wertheimer, E., (1938), J. Physiol., 92, 48.

228. Fischer, N.F., and Lackey, W.R., (1925), Am. J. Physiol., 72, 43; Stanbury, J.B., Wyngaarden, J.B., and Fredrickson, D.S., "The Metabolic Basis of Inherited Disease", 2nd ed., McGraw Hill Book Co., N.Y. (1966).

229. Cori, G.T., and Cori, C.F., (1952), J. Biol. Chem., 199, 661.

230. Schmid, R., and Mahler, R., (1959), J. Clin. Invest., 38, 1040.

231. Hers, H.G., (1959), Rev. Int. Hepat., 9, 35; ibid., (1961), Chem. Weekbl., 57, 437; ibid., (1963), Biochem., J., 86, 437.

232. Cori, C.F., Enzymatic Reactions in Carbohydrate Metabolism, Harvey Lectures, 41, 253, (1945-46); Cori, G.T., and Larner, J., (1951), J. Biol. Chem., 188, 17; Cori, C.F., Schmidt, G., and Cori, G.T., (1939), Science, 89, 464.

233. Bobbit, J.M., Periodate Oxidation of Carbohate, Adv. in Carb. Chem. Vol II, p. 3, (1965), Edited by M.L. Wolfrom and R.S. Tipson, Acad. Press Inc., Publishers, N.Y. (1956).

234. Manners, D.J., Adv. in Carb. Chem., Vol. 12, pp. 261-298 (1957).

235. Gunja, Z.H., Manners, D.J., and Maung, D., (1961), Biochem. J., 81, 392.

236. Cori, G.T., Harvey Lectures, Ser., 48, 145, (1953), Illingworth, B., (1961), Am. J. Clin. Nutrition, 9, 683.

237. Manners, D.J., and Wright, A., (1961), Biochem. J., 79, 188.

238. Larner, J., (1953), J. Biol. Chem., 202, 491.

239. Wollenberger, A., and Krause, E.G., (1963), Bio-Chim. Biophys. Acta., 67, 340.

240. Khebs, E.G., and Fischer, E.H., (1963), In Advances in Enzymology, Vol. 24, p. 263, (Ed.) F.F. Nord, N.Y. Wiley.

241. Rall, T.W., and Sutherland, E.W., (1958), J. Biol. Chem., 232, 1065.

242. Leloir, L.F., Olavarria, J.M., Goldberg, S.H., and Carminatti, H., (1959), Arch. Biochem. Biophys., 81, 508.

243. Luck, D.J.L., (1961), J. Biophys. Biochem. Cytol., 10, 195.

244. Villar-Palasi, C., and Larner, J., (1960), Arch. Biochem. Biophys., 86, 61; ibid., (1960), 86, 270.

245. Hiss, R., and Pearse, A.G.E., (1961), Proc. Soc. Exptl. Biol. Med., 107, 569.

246. Cori, G.T., and Cori, C.F., (1943), J. Biol. Chem., 151, 57.

247. Leloir, L.F., and Cardini, C.E., (1957), J. Am. Chem. Soc., 79, 6340: Leloir, L.F., and Goldenberg, S.H., (1960), J. Biol. Chem., 235, 919; Robbins, P.W., and Lipmann, F., Recent Observations on the Mechanism of Glycogen Synthesis in Muscle in Regulation of Cell Metabolism, Ciba Foundation Symposium; Wolstenholme, G.E.W., and O'Connor, C.H., (Eds.) Little, Brown and Co., Boston, p. 188, (1959), Whelman, W.J., and Cameron, M.P., (Eds.), Control of Glycogen Metabolism Cibe Foundation Symposium, pp. 115-175, 200-412, (1964), Little Brown and Co., Boston.

248. O'Brian, J.P., and Sharpe, A.R., (1965), Metabolism 14, 1294, (A Review).

249. Merrlli, J.P., Hampers, C.L., (1970), (A Review), The New England J. Med., 282, (17), 953, and ibid., (1970), 282, (18), 1014.

250. Defronzo, R.A., Andres, R., Edgar, P., Walker, W.G., (1973), Carbohydrate Metabolism in Uremia: (A review), Medicine, 52, (5), 469, Publisher, The Williams and Wilkins Co.

251. Hampers, C.L., Schupak, E., (1967), N.Y., Grune, and Stratton.

252. Nutrition in Renal Disease; Proc. Conf. at University of Manchester on June 29-30, (1967), (Eds.), G.M. Berlyne, E.S.S. Edinburgh Livingstone, Ltd. (1968).

253. Welt, L.G., Symposium on Uremia, (1968), Amer. J. Med., 44, 653.

254. Rubenstein, A.H., and Spitz, I., (1968), Diabetes, 17, 161.

255. Westervelt, F.B.Jr., and Schriener, G.E., (1962), Ann. Intern. Med., 57, 266.

256. Tchobroutsky, G., Del'Hortat, G.C., Rosselin, G., Assan, R., and Derot, M., (1965), Diabetologia, 1, 101.

257. Cerletty, J.M., and Engbring, N.H., (1967), Ann. Intern. Med., 66, 1097.

258. Meyers, V.C., and Killian, J.A., (1917), J. Biol. Chem., 29, 179.

259. Hamman, L., and Hirschman, I.I., (1917), Arch. Intern. Med., 20, 761.

260. Runyan, J.W.Jr., Hurwitz, D., and Robbins, S.L., (1955), New England, J. Med., 252, 388.

261. Wilson, J.E., Root, H.F., and Marble, A., (1951), New England, J. Med., 245, 513.

262. Carr, M.H., and Schloerb, P.R., (1960), Analyt. Biochem., 1, 221.

263. Menicheni, G.C., Gonella, M., Barsatti, G., and Giovannetti, S., (1971), Experientia, 27, 1157.

264. Balestri, P.L., Rindi, P., Biagini, M., and Giovannetti, S., (1972), Clinical Sci., 42, 395.

265. Hutchings, R.H., Hegstrom, R.W., Scribner, B.H., (1966), Ann. Intern. Med., 65, 275.

266. Sagilda, U., (1962), Acta. Med. Scan., 172, 405.

267. Hampers, C.L., Soeldner, J.S., Doak, P.B., and Merrell, J.P., (1966), J. Clin. Invest., 45, 1719.

268. Alfrey, A.C., Sussman, K.E., and Holmes, J.H., (1967), Metabolism, 16, 733.

269. Conn, J.W., (1965), New Engl. J. Med., 273, 1135.

270. Milner, R.D.G., and Hale, C.N., (1967), Diabetologia, 3, 47.

271. Hales, C.N., and Milner, R.D.G., (1968), J. Physiol., London, 194, 725.

272. Gorden, P., and Roth, J., (1969), Arc . Int. Med., 123, 237.

273. Lowrie, E.G., Soeldner, J.S., Hampers, C.L., and Merrill, J.P., (1970), J. Lab. Clin. Med., 76, 603.

274. Hampers, C.L., Soeldner, J.S., Gleason, R.E., Bailey, G.L., Diamond, J.A., and Merrill, J.P., (1968), Am. J. Clin. Nutr., 21, 414.

275. Spitz, I.M., Rubenstien, A.H., Bersohn, I., Abrahams, C., and Lowry, C., (1970), Quart. J. Med., 39, 201.

276. Wildberger, H.L., Ricketts, H.T., and Regret, L., (1963), Proc. Soc., Expt. Biol. Med., 112, 168.

277. Saglid, U., Andersen, V., and Andersen, P.B., (1961), Acta. Med. Scand., 169, 243.

278. Spergel, G., Bleicher, S.J., Goldberg, M., Adesman, J., and Goldner, M.G., (1967), Metabolism, 16, 581.

279. Westervelt, U., F.B.Jr., (1968), Am. J. Clin. Nutr., 21, 423.

280. ibid., (1969), J. Lab. Clin. Med., 74, 79.

281. Milner, R.D.G., and Hales, C.N., (1967), Biochem. J., 105, 28P.

282. Samaan, N.A., and Freeman, R.M., (1970), Metabolism, 19, 102.

283. Horton, E.S., Johnson, C., and Lebevitz, H.E., (1968), Ann. Int. Med., 68, 63.

284. Dzurin, R., and Valovicova, E., (1970), Clin. Chim. Acta., 30, 137.

285. Dosseter, J.B., and Gault, M.H., Carbohydrate Metabolism in Nephron Failure, Pub. by Charles C. Thomas, U.S.A. (1971).

286. Rubenstein, A.H., Lowy, C., Welborn, T.A., and Fraser, T.R., (1967), Metabolism, 16, 234.

287. Dzurik, R., Niederland, T.R., and Cernacek, P., (1969), Clin. Sci., 37, 409., Perkoff, G.T., Thomas, C.L., Newton, J.D., Sellman, J.C., and Tyler, F.H., (1958), Diabetes, 7, 375.

288. Davidson, M.B., Lowrie, E.G., and Hampers, C.L., (1969), Metabolism, 18, 387.

289. Cohen, B.D., Ann. Intern. Med., (1962), 57, 204, Cohen, B.D., Spritz, N., Rubin, A.L., and Lucky, E.H., (1961), Ann. Int. Med., 54, 1062.

290. Dzurik, R., and Brixova, E., (1968), Experientia, 24, 552.

291. Boucot, N.G., Guild, W.R., and Merrill, J.P., (1958), J. Physiol, 192, 301; Boucot, N.G., Nurser, I.K., and Merrill, J.P., (1960), ibid., 198, 797.

292. Cummings, N.B., and Zottu, S.M., (1960), Am. J. Physiol., 198, 1079.

293. Lucke, R.G., Dinwoodie, A.J., Linton, A.L., and Kennedy, A.C., (1964), J. Lab. Clin. Med., 64, 731.

294. Felig, P., Owen, O.E., Wahren, J., and Cahill, G.F., (1971), J. Clin. Invest., 48, 584, ibid., (1971), 50, 2703.

295. Felig, P., Pozefsky, T., Marliss, E., and Cahill, G.F., (1970), Science, 167, 1003.

296. Kahil, M.E., Parrish, J.E., Simons, E.L., and Brown, H., (1966), Diabetes, 15, 734.

297. Rosen, H., Meroney, W.H., and Levenson, S.M., (1958), Clin. Sci., 17, 633.

298. Young, G.A., and Parsons, F.M., (1966), Clin. Sci., 31, 299.

299. Berlyne, G.M., Lee, H.A., Giordano, C., De Pascale, C., and Esposito, R., (1967), Lancet, 1, 1339.

300. Young, G.A., and Parsons, F.M., (1969), Clin. Sci., 37.

301. Renner, D., and Heintz, R., (1965), Proc. Europ. Dial. Transpl. Ass., 2, 128.

302. Henkin, R.I., Levine, N.D., Sussman, H.H., and Maxwell, M.H., (1964), J. Lab. Clin. Med., 64, 79.

303. Richet, G., (1968), Some Aspects of Uric Acid Metabolism in Chronic Renal Failure: In Nutrition in Renal Disease, pp. 131-139, (Ed.), G.M. Berlyne, Pub. E. and S. Livingstone Ltd., Edinburgh.

304. Sorensen, L.B., (1960), Scand. J. Clin. Lab. Invest., 12, Suppl., 54, 1-214.

305. Zaremski, P.M., Hodgkinson, A., and Parsons, F.M., (1966), Nature, London, 212, 511.

306. Bennett, B., and Rosenblum, C., (1961), Lab. Invest., 10, 947.

307. Giordano, C., (1963), J. Lab. Clin. Med., 62, 231.

308. Giovannetti, S., and Maggiore, Q., (1964), Lancet, 1, 1000.

309. Shaw, A.B., Bazzard, F.J., Booth, E.M., Nilwarankur, S., and Berlyne, G.M., (1965), Quart. J. Med., 34, 237.

310. Franklin, S.S., Gordon, A., Kleeman, C.R., and Maxwell, M.H., (1967), J. Amer. Med. Ass., 202, 477.

311. Lonergan, E.T., and Lange, K., (1968), Amer. J. Clin. Nutr., 21, 595 (1968).

312. Ohlson, M.A., Handbook of Experimental and Therapeutic Diets Minneapolis, Burgess, (1962

313. Proudfit, F.T., and Robinson, C.H., Normal and Therapeutic Nutrition, 125th Ed., N.Y., MacMillan (1961).

314. Maddock, R.K., Bloomer, H.A., and De St. Jeor, S.W., (1968), Ann. Intern. Med., 69, 1003.

315. Kessner, D.M., and Epstein, F.H., (1965), Am. J. Physiol., 209, (1), 141-145.

316. McCance, R.A., and Morris, A.R., (1956), Quart. J. Expt. Physiol., 41, 365-385.

317. Grice, H.C., Laboratory Animal Care, 14, (6), pp. 484-488.

318. Amer. Sham/Searle, Technical Data (1972).

319. Tritium Labelled Molecule in Biol. and Medicine by Ludwig, E.F., Academic Press, N.Y., (1967), p. 71.

320. Fawcett, J.K., and Scott, J.E., (1960), J. Clin. Pathol., 13, 156.

321. Chancy, A.L., and Marback, E.P., Modifi'n of the Berthelot Reaction for Measuring Urea Nitrogen, (1962), Clin. Chem., 8, 130-2. Kaplan, A., Urea and Urinary Ammonia, Structure and Methods of Clinical Chemistry, V., pp. 245-50 (1965).

322. ESKALAB Laboratory Manual (1973).

323. Karmen, A., (1955), J. Clin. Invest., 34, 126.

324. Henry, R.J., et al., (1960), Amer. J. Clin. Pathology, 34, 381.

325. Oliver, I.T., (1955), Biochem. J., 61, 116.

326. Rosalki, S.B., (1967), Lab. and Clin. Med., 69, 696.

327. Nielsen, Land Ludvigsen, B., (1963), J. Lab. and Clin. Med., 62, 159.

328. Bessey, O.A., Lowry, O.H., and Brock, M.J., (1946), J. Biol. Chem., 164, 321.

329. Todd-Sanford Clinical Diagnosis by Laboratory Methods, Edited by I. Davidsohn, and Henry, J.B., 14th Edition, p. 146, (1969), Publishers, W.B. Saunders Co.

330. Barthelmai, N., and Czok, R., (1962), C. Klinische Wochenschrift, 40, 585.

331. Bloom, W.L., Lewis, G.T., Schumpert, Mary Z., and Shen, T., (1951), J. Biol. Chem., 188, 631.

332. Abdul-Akher, M., and Smith, F., (1951), J. Am. Chem. Soc., 73, 994.

333. Bell, D.J., (1948), J. Chem. Soc., Pt. 2, 992.

334. Manners, D.J., (1954), Ann. Repts. on Progr. Chem. (Chem Soc., London), 50, 288.

335. Heatley, N.G., (1935), Biochem. J., (London), 29, 2568.

336. Walaas, O., and Walaas, E.J., (1950), J. Biol. Chem., 187, 769.

337. Fong, J., Schaffer, F.L., and Kirk, P.L., (1953), Arch. Biochem. Biophys., 45, 319.

338. Meyer, K.H., Noetling, G., and Bernfeld, P., (1948), Helv. Chem. Acta., 31, 103.

339. Hers, H.G., (1959), Intern. Hepatol., 9, 35., Hers, G.H., Verhue, W., and Van Hoof, F., Europ., (1967), J. Biochem., 2, 257.

340. Larner, J., Schlischfeld, L.H., (1956), Biochem. Biophys. Acta., 20, 53.

341. Russell, J.C., Tougas, D., and Taylor, A.N., (1970), Clin. Chemistry, 16, 900.

342. Fischer, E.H., Hund, S.S., Koh, P., Seery, V.L., and Teller, D.C., (1968): Phosphorylase Relation of Structure to Activity in Control of Glycogen Metabolism; Whelan, W.J., Ed., Acadian Press, London, (1968), p. 19.

343. Zinkham, W.H., Lenhard, R.E., and Childs, B., (1958), Bull. Johns. Hopkins Hospital, 102, 169, Zinkham, W.H., and Lenhard, R.E., (1958), J. Pediat, 55, 319.

344. Gomori, G., In Colowick and Kaplan, (Eds.), Method in Enzymology, Vol. 1, N.Y., (1955), p. 138, and Gomori, G., (1948), Proc. Expt. Biol. (N.Y.), 68, 354.

345. Dole, V.P., and Mienertz, H., (1960), J. Biol. Chem., 235, (9), 2595.

346. Bligh, E.G., and Dyer, W.J., (1959), Can. J. Biochemistry and Physiol., 37, (8), 911.

347. Barion, H., and Shafrir, E., (1965), Isr. J. Med. Sci., 1, 365.

348. Thomas, L.P., and Shirly, H., (1969), Clin. Chem. Acta., 25, 55.

349. Greenwood, C.T., Adv. in Carbohydrate Chemistry, 7, 289, (1952), ibid., 11, 387 (1956).

350. Kokot, F., Kuska, J., Kozlak, H., and Zazgomik, J., (1967), Acta. Med. Pol., 9, 461.

351. Rigg, G.A., and Bercu, B.A., (1967), New Eng., J. Med., 277, 1139.

352. Maier, K.P., Hoppe-Sayler, G., Talke, H., Frochlich, J., Scholimeyer, P., and Gerok, W., (1973), Europ. J., Clin. Invest., 3, 201.

353. Perkoff, G.T., Thomas, C.L., Newton, J.D., Sellman, J.C., and Tyler, F.H., (1958), Diabetes, 7, 375.

354. B.M.C. Bulletin on CPK: Boehringer Mannheim Corporation, 219 East 44th St., N.Y. 10017.

355. Monitor Azurechrome Bulletin, American Monitor Corporation, Indianapolis, Inidana, H6268.

356. David, D.S., Sakai, S., Granda, J., Chigh, J.S., Riggio, R.R., Stenzel, K.H., and Rubin, A.L., (1973), Trans. Amer. Soc. Artif. Int. Organs, Vol. XIX, 440.

357. Russell, R.G.G., Bisaz, S., and Fleisch, H., (1969), Arch. Int. Med., 124, 571.

358. Deluca, H.F., (1971), Recent Prog. in Hormone Res., 27, 479.

359. Russell, R.G.G., Bisaz, S., Donath, P., Morgan, D.P., and Fleisch, H., (1971), J. Clin. Invest., 50.

360. Jung, A., Russell, R.G.G., Bisaz, S., Morgan, D.G., and Fleisch, H., (1970), Amer. J. Physiol., 218, 1757.

361. Gothlin, G., and Friesson, J.L.E., (1971), Isr. J. Med. Sci., 7, 488.
Vaes, G., and Vorevan, J., (1971), ibid. 7, 401.

362. Siddiqui, J., and Kerr, D.N.S., (1971), Brit. Med. Bull., 27, 153.

363. Fleisch, H., and Russell, R.G.S., (1972), Dent. Res., (suppl), 51, 324.

364. Bombar, H., Krempner, K., Schmitt, E., Holtz, M., Klinkmann, H., (1973), Europ. Dialys. Transpl. Assoc., p. 43.

365. Westerman, M.P., Jenkins, J.L., Dekker, A., et al., (1967), Lancet, ii, 755; Murphy, G.P., Mirand, E.A., and Grace, J.T., Jr., (1969), Annals. of Surgery, 170, 581.

366. Gral, T., Schroth, P., and Palma, J.R.De., (1970), Proc. Europ. Dialysis and Transpl. Assoc., VII, 359, (1970), (Editor) Cameron, J.S., Pitman Medical and Scientific Publishing Co., Ltd.

367. Maokenzie, J.C., Ford, J.E., Waters, A.H., Harding, N., Catteli, W.R., and Anderson, B.B., Proc. Europ. Dialys. Transpl. Assoc. V, 172 (1968), (Ed.) Kerr, D.N.S., Excerpta. Medica. Foundation.

368. John, F.S., Robert, R.R., Ghoong, S.C., Kurt, H.S., Albert, L.R., (1973), Am. Soc. Artif. Int. Org., XIX, 63.

369. Weisinger, J.R., Reaven, G.M., Coplon, N.S., and Swenson, R.S., (1973), Trans. Amer. Soc. Artif. Int. Organ, XIX, 277.

370. Meinert, C.L., (1972), Diabetes, 21, 1197.

371. Hills, J.B., and Kessler, G., (1961), J. Lab. Clin. Med. 57, 970.

372. Free, A.H., and Free, H.M., (1972), in Uro-Dynamics, Ames Company, Indiana.

373. Landau, B.R., Leonards, J.R., (1960), Amer. J. Physiol., 198, 793.

374. Gavosto, F., Pileri, A., and Brusca, A., (1957), Biochem. Biophys. Acta., 24, 250.

375. Eischied, A.M., and Kochakjan, C.D., (1954), Proc. Soc. Exptl. Biol. Med., 85, 339 (1954).

376. Blecher, M., and White, A., (1957), Federation Proc., 16, 155.

377. Scientific Table by Geigy, p. 32, 7th Edition, (Editors) Dien, K., and Lentner, C., Published by J.R. Geigy, A.G., Basle, Switzerland.

378. In Biological Chemistry by Mahler, H.R., and Cordes, E.H., (1966), Pub. by Harper and Row, N.Y.

379. Pflüger, E., Pflüger's Arch. Ges. Physiol., (1903), 96, 1.

380. Carroll, N.V., Longley, R.W., and Roe, J.H., (1956), J. Biol. Chem., 220, 583.

381. Stelten, M.R., Katzen, H.M., and Stelten, D., Jr., (1958), J. Biol. Chem., 232, 475.

382. Willstatter, R., and Rholdewald, (1934), Ztschr. Physiol. Chem., 225, 103.

383. Kitsvan Heijningen, A.J.M., and Kemp, A., (1955), Biochem. J., 59, 487.

384. Kemp, A., and Kits, Van Heijningen, A.J.M., (1954), Biochem. J., 56, 646.

385. Lourau, M., and Meyer, F., (1948), J. Physiol., 50, 5.

386. Androuny, G.A., and Russell, J.A., (1956), Endocrinology, 59, 241.

387. Bloom, W.L., and Knowlton, G.C., (1953), Am. J. Physiol., 173, 545.

388. Russell, J.A., and Bloom, W.L., (1955), Am. J. Physiol., 183, 345.

389. Hanson, R.W., Schwartz, H.S., and Barker, S.B., (1960), Am. J. Physiol., 198, (4), 800.

390. Farquhar, J.W., Frank, A., Gross, R.C., and Reaven, G.M., (1966), J. Clin. Invest., 45, 1648.

391. Gershberg, H., Hulse, M., and Javier, Z., (1968), Obstet Gynec., 31, 186.

392. Hazzard, W.R., Spiger, M.J., Bagade, J.D., and Bieman, E.L., (1969), New Eng. J. Med., 80, 471.

393. Sachs, B.A., Wolfman, L., and Herzig, D., (1969), Obstet, Gynec., 34, 530.

394. Reaven, G.M., Lerner, R.L., and Stern, M.P., (1967), J. Clin. Invest., 46, 1756.

395. Ford, F.D., Jr., Bozian, R.C., and Knowles, H.G., Jr., (1968), Amer. J. Clin. Nutr., 21, 904.

396. Symposium on Uremic Toxin: (Eds.): Welt, L.G., Black, H.R., and Kruger, K.K., (1970), Arch. Intern. Med., 126, 791.

397. Baxter, J.H., (1962), Arch. Intern. Med., 109, 742.

398. Marsh, J.B., and Drabkin, D.L., (1960), Metabolism, 9, 948.

399. Marsh, J.B., and Drabkin, D.L., (1955), J. Biol. Chem., 212, 633.

400. Burns, L.C., Mechal, S.K., and Koeppe, R.E., (1970), Life Sci., 9, pt. II, 1045.

401. Betheil, J.J., Feigelson, M., and Feigelson, P., (1965), Biochem. Biophys. Acta, 104, 92.

402. Cohen, P.P., (1937), J. Biol. Chem., 119, 333.

403. Lorber, W., Lifson, N., Wood, H.G., Sakami, W., and Shreeve, W.W., (1950), J. Biol. Chem., 183, 517.

404. Mayer, M., Brenner, T., and Shafrir, E., (1971), Isr. J. Chem., 9, 37, BC.

405. Asatoor, A.M., and Armstrong, M.D., (1967), Biochem. Biophys. Res. Comm., 26, 168.

406. Ichihara, A., and Greenberg, D.M., (1955), Proc. Natl. Acad. Sci. U.S., 41, 605.

407. Lien, O.G., Jr., and Greenberg, D.M., (1953), J. Biochem., 200, 367.

408. Dent, C.N., (1947), Science, 105, 335.

409. Matsuo, Y., and Greenberg, D.M., (1955), J. Biol. Chem., 215, 547.

410. Barsook, H., Deasy, C.L., Haagen, Smit, A.J., Keighley, G., and Lowy, P.H., (1948), J. Biol. Chem., 176, 1383 and 1395.

CHAPTER VIII

APPENDICES

APPENDIX A

ABBREVIATIONS USED

BUN - Blood urea nitrogen
PAP - Plasma alkaline phosphatase
EDTA - Ethylenediamine tetra acetic acid
NM - nanometer = mμ
M - molar
N - normal
A - absorbance (= O.D. = optical density)
ΔA - change in absorbance
ε - molar absorption co-efficient
M - concentration in moles/liter
TV - total volume
SV - sample volume
E - enzyme
S - substrate
SGOT - serum glutamate oxaloacetate transaminase (or serum-aspartate aminotransferase)
CPK - creatine phosphokinase
G-6-PDH - glucose-6-phosphate dehydrogenase
G-6-P - glucose-6-phosphate
ATP - adenosine triphosphate
ADP - adenosine diphosphate
NAD$^+$ - nicotinamide adenine dinucleotide oxidized form (previously DPN$^+$)

ABBREVIATIONS USED (Continued)

NADH$_2$ - nicotinamide adenine dinucleotide reduced form (previously DPNH)

NADP$^+$ - nicotinamide adenine dinucleotide phosphate (oxidized form) (previously TPN$^+$)

NADPH$_2$ - nicotinamide adenine dinucleotide phosphate) (reduced form) (previously TPNH)

MDH - malic dehydrogenase

IU - international unit

HK - hexokinase

H$_{ct}$ - hematocrit

POPOP - 1,3-bis 2(-5-phenyloxazolyl)-benzene

PPO - 2,5-diphenyloxazole

TCA - trichloro acetic acid

rt - room temperature

SO - Sham operated rat

U - uremic rat

RD - restricted diet rat

OD - optical density

UDPG - uridine - 5- D-glucosyl pyrophosphate)

UDP - uridine diphosphate

UTP - uridine triphosphate

G-1-P - glucose-1-phosphate

PP$_i$ - inorganic pyrophosphate

P$_i$ - inorganic phosphate

ABBREVIATIONS USED (Continued)

5-AMP - 5-adenosine monophosphate

AGPA - aminoguanidino propionic acid

SSA - sulfosalicyclic acid

DVV - divinylbenzene

ppt - precipitate

FFA - free fatty acid

dpm - disintegration per minute

cpm - count per minute

CE - counting efficiency

µCi - millicurie (2.22 x 10^6 dpm)

mCi - millicurie (2.22 x 10^9 dpm)
Ci - currie (3.7 x 10^{10} dps ≡ 2.22 x 10^{12} dpm)

dps - disintegration per second

LDH - lactate dehydrogenase

GFR - glomerular filtration rate

CRF - chronic renal failure

ARF - acute renal failure

g.i.t. - gastro intestinal tract

PTH - parathyroid hormone

SDH - serine dehydratase

TPO - tryptophan oxygenase

ETKA - erythrocyte transketolase activity

ACTH - adreno corticotropic hormone

EP - erythropoietin

ABBREVIATIONS USED (Continued)

RNA - ribonucleic acid

ADH - anti-diuretic hormone

ULDH - urinary lactic dehydrogenase

UAP - urinary alkaline phosphatase

UGOT - urinary glutamic oxaloacetic transaminase

PSP - phenolsulfonphthalein

PAH - para-amino-hippurate

C_{PAH} - PAH clearance

RPF - renal plasma flow

C_{in} - clearance of insulin

GFR - glomerular filtration rate

IVP - intravenous pyelography

U_{osm} - urine osmolality

GSA - guanidinosuccinic acid

GAA - guanidino acetic acid

TCA - tricarboxylic acid cycle

PEPCK - phosphoenol pyruvate carboxy kinase

ITP - inosine triphosphate

GTP - guanosine triphosphate

FDP - fructose diphosphate

PFK - phosphofructokinase

FDPP - fructose diphosphate phosphatase

F-1,6-dPase - frucotse, 6-diphosphatase

OAA - oxaloacetic acid

PMG - phosphoglucomutase

GLDH - glutamate dehydrogenase

ABBREVIATIONS USED (Continued)

CA - carbonic anhydrase

PCT - proximal convoluted tubules

dct - distal convoluted tubules

LAP - leucine amino peptidase or amino acid arylamidase

GK - glucokinase

PK - pyruvate kinase

GPT - glutamic pyruvic transaminase

AAT - aspartate amino transferase

ALAT - alanine amino transferase

TAT - tyrosine amino transferase

APPENDIX B

STATISTICAL METHODS

The results are expressed as the mean ± S.D. (standard deviations) for samples obtained in every two week period; and mean ± S.E. (standard errors) for the pooled values obtained from samples of 1 to 9 weeks and 10 to 19 weeks periods, respectively.

Means, standard deviations, standard error of the means, F test, and t test (confidence limit of the mean) for two sets of data were computed using the computer program (focal format) designed and programmed by Mr. C. Ediss, and using the Digital computer PDP//8L. The program description and the working formula [obtained from "An Introduction to the Experimental Method" by Maxwell Little, published by Burgess Publishing Co., Minn., (1961), P. 29-31, equation 5.3], and the C-Focal, 69 CE format are shown on pages 311-313.

Comparison of 2 Sample Means by 't' test

The 't' value is a ratio of the difference in means to the standard error of that difference and can be applied to compare any two samples data. The calculated 't' value is compared with the tabulated values for 't' under the indicated level of one tail or two tails test or both (377) to see if two samples originate from the same population.

STATISTICAL METHODS (Continued)

If the calculated 't' was equal to or greater than those tabulated, it can be concluded that the two sample means did not come from the same population; i.e., the null hypothesis (which assumes that both samples are taken from a single population) is rejected but if the calculated 't' is smaller than the tabulated 't', then the null hypothesis is accepted.

All 'p' values reported are on a one tail test basis.

Title: Means, Standard Deviations, Standard Error of the Means, F Test and t Tests for two Sets of Data

Author: C. Ediss Date: July, 1971 Format: Focal

Program Description: The program accepts two sets of data sequentially. Numbers are terminated by a space, and then the program asks for a test response. If the previous number on that line is okay, type a zero and a space; if an error has been made, respond with -1 and a space and the program will ignore that particular input number; after the last number in a set of data respond with a one and a space, and the program proceeds.

STATISTICAL METHODS (Continued)

The formulae used are as follows:

$$\bar{x} = \frac{\Sigma x}{n} \; ; \quad \sigma_x = \frac{n\Sigma x^2 - (\Sigma x)^2}{n(n-1)} \; ; \quad \sigma_x = \frac{\sigma x}{n}$$

$$F = \frac{\sigma_{x_1}}{\sigma_{x_2}} \; ; \quad df_1 = n_1 - 1; \quad df_2 = n_2 - 1; \quad \text{sorted to give } F > 1$$

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\dfrac{n_2[n_1(\Sigma x_1{}^2) - (\Sigma x_1)^2] + n_1[n_2(\Sigma_{x_2}{}^2) - (\Sigma x_1)^2]}{n_1 n_2 (n_1 + n_2 - 2)} \; \dfrac{n_1 + n_2}{n_1 n_2}}}$$

$$df = n_1 + n_2 - 2$$

from 'Introduction to Experimental Method' Maxwell Little.

```
 F A
*
*C-FOCAL,69CF
*
*01.01 E
*01.03 T !!"FOLLOW COLONS WITH DATA";
*01.05 F J=1,2;S I=0;S B=0;S C=0;D 3
*01.10 T !!"                 FIRST SET OF DATA       SECOND SET OF DATA"!!
*01.15 T "NO OF DATA          "%3,N(1),"                                ",N(2),!!!
*01.20 F I=1,2;S M(I)=S(I)/N(I);S D(I)=N(I)*C(I)-S(I)↑2
*01.25 T "  MEAN          "%,M(1),"                 ",M(2),!!;S DM=M(1)-M(2);
*01.30 F I=1,2;S V(I)=FSQT(D(I)/(N(I)*(N(I)-1)))
*01.35 T "STANDARD"!"DEVIATION     ",V(1),"               ",V(2);
*01.36 T !!"STANDARD";
*01.40 S K=N(1);S L=N(2);
*01.45 T !"ERROR OF       ",V(1)/FSQT(K),"                 ",V(2)/FSQT(L);
*01.50 T !"THE MEAN"!;
*01.55 S F=V(1)↑2/V(2)↑2;I (F-1)1.6;S ZA=K;S ZB=L;G 1.65;
*01.60 S F=1/F;S ZA=L;S ZB=K;
*01.65 T !!" F ",F,"   DF1 "%3,ZA-1,"   DF2 ",ZB-1,!;
*01.70 S T=DM/FSQT(((L*D(1)+K*D(2))/((K*L)↑2*(K+L-2)))*(K+L));
*01.80 T !" T "%,FABS(T),"   DF "%3,L+K-2,!!!!!;Q;
*
*03.05 S I=I+1
*03.06 A !X,P;
*03.07 I (P)3.06;S B=B+X;S C=C+X↑2;I (-P)3.1;G 3.05;
*03.10 S S(J)=B;S C(J)=C;S N(J)=I;T !"*";R;
**
*
*
*
*C          ANALYSIS OF TWO SETS OF DATA
*
*C      INDICATE END OF A NUMBER BY A SPACE
*
*
*C          INPUT DATA TO BE FOLLOWED BY :-
*
*C        A ZERO & A SPACE IF DATA OK
*C        -1 & A SPACE IF NOT. THAT DATA THEN IGNORED
*C        A ONE & A SPACE AT THE END OF EACH SET OF DATA
*
*
*
*
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APPENDIX C

1. Introduction

(a) Unit of Enzyme Activity

In 1961 the Commission on Enzymes of the International Union of Biochemistry recommended the use of a standard unit for all enzymes. "One IU of any enzyme is that amount which will catalyze the transformation (reaction) of one micromole of substrate per minute per liter under defined conditions of temperature, pH, buffer system, substrate and co-factor concentrations". The temperature suggested in 1961 to be 25°C has been revised in the second report to be 30°C.

Therefore, it is a must to state at which temperature the determination of enzyme activities has been performed.

The "optical test" introduced by Otto Warburg in 1936 is based on the fact that the reduced nicotinamide adenine dinucleotides $NADH_2$ and $NADPH_2$ absorb light with a peak at 340 nm, while the oxidized forms NAD and NADP show no absorption between 300 and 400 nm; they absorb at 260 nm. Any dehydrogenase reaction in which either NAD or NADP are reduced or $NADH_2$ or $NADPH_2$ oxidized, can be measured by recording the increase or decrease, respectively, of the absorbance at 340 nm or a close-by wave length.

APPENDIX C (Continued)

Knowing the molar absorption co-efficient (ε) and light path (d = 1.0 cm) (inside distance between the cell walls of a square cuvette or the inner diamter of round cuvettes) the concentration of NAD or NADP reduced or $NADH_2$ or $NADPH_2$ oxidized can be measured from the equation:

$$A = \varepsilon \times c \times d \quad \text{or} \quad C = \frac{A}{\varepsilon \times 1.0} = \text{moles/liter}$$

(b) Computation of Enzyme Units

Units of enzyme activity are computed with reference to a reaction product. The computation can be based on a "standard", by preparing a solution with an exactly known concentration of the reaction product. An aliquot of the standard solution is treated and measured together with the sample.

A standard is not required if the molar absorption co-efficient, ε, of a reaction product is known at a given wave length as, for example, for NADH (and NADPH).

Calculation based on the molar extinction co-efficient of a reaction product can be made:

i. with absorbance and/or

ii. tan α

For example,

i. with the absorbance

APPENDIX C (Continued)

$$\frac{A\ \text{Sample}}{\varepsilon \times d} \times 10^3 \times \frac{1}{T} \times \frac{TV}{SV} = \text{IU } (\mu\text{mole/min/l})$$

where ε = molar extinction co-efficient

d = light path (diameter of the cuvette in cm)

TV = total reaction volume in ml

SV = sample volume in ml

ii. with tan α:

When time reaction curves are recorded, the angle (α) between the curve and the abscissa can be used to calculate the enzyme activity in the general formula:

$$\text{IU at } 37°\text{C} = \frac{\Delta A}{T} \times \frac{TV}{SV} \times \frac{1}{\varepsilon . d} \times 1000$$

where ΔA = absorbance change between two readings.

2. SGOT

One unit of SGOT is defined as that amount of enzyme activity which will cause a change in $NADH_2$ absorbance (A) at 340 nm of 0.001 A/ml of serum per minute in a total volume of 3.0 ml in a cuvette with a 1 cm light path. Spectrophotometric unit at 37°C was calculated as follows:

(For 10 µℓ sample) SGOT units/ml = ΔA x 6665

where ΔA = absorbance 1 (at 10 minutes)
minus absorbance 2 (at 25 minutes)
ΔA/minute was obtained by dividing ΔA by 15.

APPENDIX C (Continued)

The factor was derived from the general formula as follows:

$$\text{SGOT units/ml} = \frac{\Delta A}{T} \times \frac{1}{\text{Sample size in ml}} \times 1000$$

$$= \frac{\Delta A}{15} \times \frac{1}{0.01} \times 1000 = \Delta A \times 6665$$

The factor 6665 was obtained as by using 50 μℓ sample which gave ΔA x 1333. For 10 μℓ this factor is 5 x. Spectrophotometric units can be converted into SGOT IU at the same temperature as follows:

SGOT IU (μmoles/min/l) = spectrophotometric units x 0.48

(Since 0.001 A change per ml serum in total volume of 3 ml this would be equivalent to 1A change per liter in TV of 3 liters. Since, $\varepsilon = 6.22 \times 10^6$ cm^2/mole, the conversion factor = $\frac{3}{6.22} = 0.48$).

3. CPK

One IU of CPK is defined as that amount of enzyme which transfers one micromole of phosphate per minute per liter of serum at 37°C. (For 10 μℓ sample):

IU CPK = ΔA x 4823

where ΔA = absorbance change. Between readings at 10 and 15 minutes. ΔA/1 minute was derived by dividing ΔA by 5. The factor is derived from the general formula:

APPENDIX C (Continued)

$$\text{IU @ } 37^\circ\text{C} = \frac{\Delta A}{T} \times \frac{TV}{SV} \times \frac{1}{10^{-6}\varepsilon} \times 1000$$

where ε = molar absorptivity of $NADPH_2$ at 340 nm
= 6.22×10^6 cm^2/mole

T = time between absorbance readings
(=5 minutes)

ΔA = absorbance change between readings

SV = sample volume in ml
(= 0.01 ml)

TV = total reaction volume in ml
(= 1.50 ml)

By substitution:

$$\frac{\Delta A}{5} \times \frac{1.50}{0.01} \times \frac{1}{6.22 \times 10^{-6} \times 10^{6}} \times 10{,}000 = \frac{\Delta A \times 150{,}000}{31.10}$$

or:

$$\Delta A = \frac{1{,}500{,}000}{311} = 4823$$

4. Alkaline Phosphatase

One IU of alkaline phosphatase activity is defined as that amount of enzyme activity which will catalyze the reaction of one micromole of substrate/liter plasma/minute and calculated as follows:

APPENDIX C (Continued)

(For 10 µℓ sample at 37°C) $\text{IU/liter/min} = \frac{\Delta A}{\text{min}} \times 1695$

where ΔA/min = absorbance change between two readings at 5 and at 15 minutes and ΔA divided by 10 the factor 1695 was derived from the general formula:

$$\text{IU/liter} = \frac{\Delta A}{T} \times \frac{TV}{SV} \times \frac{1}{10^{-3}\varepsilon} \times \frac{1000}{SV}$$

where ε = molar absorptivity of p-nitrophenol

= 17.7×10^3 at 415 nm

TV = 3.0

SV = 0.01 ml

Substituting:

$$\text{IU/liter} = \frac{\Delta A}{10} \times \frac{3.0}{17.7 \times 10^{-3} \times 10^3} \times \frac{1000}{0.01}$$

or:

$$= \Delta A = \frac{300{,}000}{177} = 1695 \text{ (at } 37°\text{C)}$$

5. Amylo-1,6-Glucosidase

The activity of the enzyme was expressed in two ways:

(a) total activity in µmole glucose/g tissue/hour.

APPENDIX C (Continued)

(b) Hers units (i.e. 0.1% of ^{14}C-glucose added/per g tissue per minute. All calculations were made by computer, a speciment is as follows:

i. Specific activity of ^{14}C-glucose

$= 240$ mc/mM

Therefore, 0.1 ml substrate

$\equiv 5.32 \times 10^4$ dpm

$\equiv 1$ μM glucose

0.1 ml substrate after reacting with 0.1 ml tissue homogenate gave 2828 cpm

$$\text{which} \equiv \frac{2828 \times 100}{56} = 5.05 \times 10^3 \text{ dpm on}$$

$$\frac{5.05 \times 10^3}{5.32 \times 10^4} \equiv 9.50 \times 10^{-2} \text{ μmole},$$

where 56 is the counting efficiency in percent.

This was in 2.57 mg tissue with a reaction time of 1 hour. Therefore, the total enzyme activity per g per minute:

$$= \frac{9.5 \times 10^{-2}}{2.57 \times 10^{-3}} = 37 \text{ μmole glucose/minute/g}$$

ii. one unit (Hers) $\equiv$ 0.1% of 5.54×10^6 dpm used per 0.1 ml substrate. This is equal to $\frac{0.1}{100} \times 5.54 \times 10^6 = 5540$ dpm $\equiv$ 1 unit

$$5540 \text{ dpm} \equiv 5540 \times 0.56 = 3102 \text{ cpm}$$

To convert observed cpm into units, cpm was divided by 3102 and the unit was divided by the g of tissue used for the one hour reaction between substrate (0.1 ml) and homogenate (0.1 ml) at 37°C. Finally, the unit was expressed per g tissue per minute, for example, one reaction gave 41740 cpm which:

$$\equiv \frac{41740}{3102} = 13.5 \text{ units (Hers).}$$

This was in 0.1 ml homogenate of 4.08 mg tissue in 1 hour of incubation time. Therefore, unit per g tissue per minute:

$$= \frac{13.5}{4.08 \times 10^3 \times 60} = 55 \text{ units (Hers)}$$

6. Glycogen Synthetase "I" and "D"

The enzyme activity is expressed in µM glucose/g/minute. The results were calculated by using a computer program. The specific activity of the UDPG-^{14}C used was 5.06×10^{11} dpm/mM. Therefore, 0.1 ml of this has 1 µM equivalent of glucose in 5.06×10^4 dpm. For example, using 0.1 ml substrate and 0.1 ml tissue homogenate (which is equivalent to 5.68 mg tissue) and a reaction time of 15 minutes, we obtained 3153 cpm which:

$$\equiv \frac{3153}{56} \times 100 = 0.56 \times 10^4 \text{ dpm}$$

Therefore, 0.56×10^4 dpm

$$\equiv \frac{0.56 \times 10^4}{5.06 \times 10^4} \text{ dpm} \equiv 0.11 \ \mu\text{M}$$

This is in 5.68 mg, therefore, activity per g per minute:

$$= \frac{0.11}{5.68 \times 10^{-3} \times 15} = 1.30 \ \mu\text{M glucose.}$$

7. α-Glucan Phosphorylase "a" and "b"

The enzyme activity is expressed as µM glucose per g tissue per minute. The computer program was used throughout for calculation of results. The specific activity of G-1-P-^{14}C was 4.8×10^8 dpm/mM. Therefore, 0.1 ml of this solution has one µM of glucose (4.8×10^4 dpm). Therefore, the cpm was corrected for recovery and counting efficiency and then divided by 4.8×10^4 to obtain µM of glucose. This was then expressed as per g of tissue per minute. For example, 0.1 ml substrate reacting with 0.1 ml tissue homogenate in five minutes gives the count of 58,951 cpm in the glycogen synthesized. So,

$$58{,}951 \text{ cpm} = \frac{58{,}951 \times 100}{56} = 10.53 \times 10^4 \text{ dpm}$$

$$10.53 \times 10^4 \text{ dpm} \equiv \frac{10.53 \times 10^4}{4.8 \times 10^4} = 2.19 \ \mu\text{M}$$

This is in 6.17 mg of tissue. Therefore, the enzyme activity per g of tissue per minute:

$$= \frac{2.19}{6.17 \times 10^{-3} \times 5} = 71\ \mu\text{M glucose}$$

8. Glucose-6-PDH

An international unit of (IU) of G-6-PDH is that amount of enzyme which oxidizes 1 μM of G-6-P per minute per liter of serum. To calculate IU of G-6-PDH subtract the two minute reading from 7 minute reading and multiply the resulting ΔA by the appropriate factor obtained from the following formula in case of liver 20,000 x g supernatant and expressed in IU/liter liver 20,000 x g supernatant per minute.

$$\text{IU} = \frac{A}{T} \times \frac{TV}{SV} \times \frac{1}{\varepsilon \times 10^{-6}} \times 100$$

where ΔA = absorbance change between readings

T = time betweeh absorbance readings

SV = sample volume in ml

TV = total reaction volume in ml

= reagent volume and sample volume

ε = molar absorptivity of $NADPH_2$ at 340 nm

= $6.22 \times 10^{6}\ cm^{2}$/mole

Then IU per liter of liver supernatant per minute was divided by the amount of tissue used to obtain one liter

APPENDIX C (Continued)

of 20,000 x g supernatant and finally the enzyme activity was expressed in IU/g liver/minute. For example, a factor of 9680 was obtained for a sample of 10 lambda as follows:

$$IU = \frac{\Delta A}{5} \times \frac{3.01}{.01} \times \frac{1}{6.22 \times 10^{6} \times 10^{-6}} \times 1000$$

$$= \Delta A \times 9680 \text{ per liter per minute}$$

Now to express IU in per g liver per minute, this factor ΔA x 9680 should be divided by the amount of liver present in one liter of supernatant. For example, using 416 g liver in one liter of supernatant with a 10 lambda sample, it gave ΔA = 0.237. Therefore, IU per g liver per minute = $\frac{0.237 \times 9680}{416} = 5.52$.

APPENDIX D

1. BUN Calculations

(a) Reagents Used

i. EDTA, one percent, pH 6.5

ii. Buffered urease (Sigma type II); Sigma Chemicals Ltd., (St. Louis, Miss., U.S.A.)

Dissolve 50 mg of urease in a few ml of one percent EDTA in a 100 ml volumetric flask. Add 0.5 ml of glycerol and make up to 100 ml with one percent EDTA.

iii. Phenol Color Reagent

Dissolve 10 g of reagent grade phenol in distilled water and add 0.05 g of sodium nitroprusside and dissolve. Make up to one liter and store in a dark bottle.

iv. Alkaline Hypochlorite Reagent (NaOCl)

Dissolve 5 g of sodium hydroxide in approximately 500 ml of distilled water. Add 8 ml of commercial sodium hypochlorite solution (five percent available chlorine). Make up to one liter.

v. Stock Urea Standard

Dissolve 2.143 g of reagent grade urea in distilled water and dilute to 100 ml. This gives 1 ml = 10 mg N.

vi. Working Urea Standards

Dilute each of the following amounts of stock urea standard to 10 ml with distilled water: 0.10 ml,

APPENDIX D (Continued)

0.30 ml, and 0.50 ml. This gives standards of 10, 30, and 50 mg N/100 ml, respectively.

vii. Control Serum and Labrol

(b) Procedure

i. Pipette 0.50 ml of buffered urease standard into each of a series of test tubes for a blank, three standards, control serum and unknowns.

ii. To the appropriate tube (except the blank) add 20 lambda of standard, control serum, or unknown.

iii. Place the test tubes in a 50°C water bath for six minutes or 37°C bath for 15 minutes.

iv. Add to each tube (including the blank) in the following order:

a. 5 ml phenol color reagent

b. 5 ml alkali hypochlorite reagent.

Promptly mix the contents of each test tube using parafilm as a cover for the tubes to prevent contamination.

v. Replace the test tube in the 50°C water bath for six minutes or 30°C for 15 minutes for color development.

vi. Read the absorbance on a photometer using a wavelength of 540 nm. The color is stable for at least five hours.

(c) Calculations

i. Read from a Standard Graph

APPENDIX D (Continued)

To draw the graph, plot the photometer readings (as ordinate) against the urea nitrogen concentrations (as abscissa). The standard graph should yield a straight line.

ii. $$\frac{\text{Reading of Unknown}}{\text{Reading of Standard}} \times \text{Concentration of standard}$$

$$= \text{mg N/100 ml}$$

(d) Precautions

i. The procedure is highly sensitive to ammonia and consequently should not be used where ammonia contamination is likely to occur.

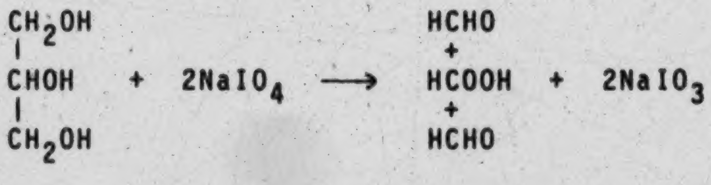
ii. The blank should not exceed 40 Klett units when read against water; if it exceeds this value, the reagents either have become contaminated or have aged.

iii. A highly turbid specimen which may impart turbidity to the final colored solution should be corrected by adding phenol color reagent to specimen blank. This situation may only occur in case of analysis of highly lipemic specimens.

iv. Urea nitrogen concentrations as high as 50 mg per 100 ml in the sample can be determined without dilution. If the color is too intense to read accurately, dilute the unknown with the blank (e.g. 1 ml unknown, 2 ml blank) and apply the appropriate dilution factor (x 3 in this sample).

2. Structure Determination of Glycogen

Sodium thiosulfate and sodium periodate (Allied Chemicals, N.Y.) both were standardized by the oxidation of glycerol (a standard compound) with periodate (Fisher Scientific Co., Fairlawn, N.J.). When treated with KI and H_2SO_4, the sample containing glycerol liberates two moles less I_2 (per mole of glycerol) than the corresponding blank, the I_2 being measured by titration with thiosulfate.



$$\begin{array}{c} \mathrm{CH_2OH} \\ | \\ \mathrm{CHOH} \\ | \\ \mathrm{CH_2OH} \end{array} + 2\mathrm{NaIO_4} \longrightarrow \begin{array}{c} \mathrm{HCHO} \\ + \\ \mathrm{HCOOH} \\ + \\ \mathrm{HCHO} \end{array} + 2\mathrm{NaIO_3}$$

The moles of glycerol were calculated; 1 mole of glycerol $\equiv$ 2 moles of I_2 $\equiv$ 4 moles of thiosulfate or 2 moles of thiosulfate $\equiv$ 1 mole of I_2 reduced to IO_3^-. Therefore, 1 ml of 0.1 M thiosulfate $\equiv$ 0.5 ml of 0.1 M iodate.

(a) Periodate Consumptions
(Uremic Sample)

We know that four moles of thiosulfate reacts with 2 moles of I_2 generated by 2 moles of $IO_4^- \rightarrow IO_3^-$ (1 mole IO_4^- = 2 moles thiosulfate). Therefore, moles of reacted IO_3^- = moles of $Na_2S_2O_3/2$.

The difference between blank and sample (2 ml) = $12.6 \sim 11.6 = 1$ ml. Therefore, number of moles of thiosulfate $= 1 \times 0.1 \times 10^{-3}$

$= 1 \times 10^{-4}$ moles.

Therefore, moles of 10^{-4}

$$= \frac{1 \times 10^{-4}}{2}$$

$= 5 \times 10^{-5}$ moles.

Total IO_4^- consumption in 47 ml (40 ml 0.46% glycogen + 7 ml $NaIO_4$):

$$= \frac{5 \times 10^{-5} \times 47}{2} = 1.18 \times 10^{-3} \text{ moles}$$

40 mls 0.46% glycogen

$$= \frac{0.46 \times 40}{100} = 0.184 \text{ g glycogen}$$

Therefore, 1 g glycogen (of uremic sample) consumes

$$\frac{1.18 \times 10^{-3}}{0.184} = 6.41 \times 10^{-3} \text{ moles of } IO_4^-$$

Similarly, control; difference between blank and sample

$= 11.4 - 12.5 - 1.1$ ml thiosulfate

Number of moles of thiosulfate

$= 1.1 \times 0.1 \times 10^{-3} = 1.1 \times 10^{-4}$ moles

of $IO_4^- = \dfrac{1.1 \times 10^{-4}}{2} = 5.5 \times 10^{-5}$ moles.

APPENDIX D (Continued)

Total IO_4^- consumption in 47 ml (40 ml, 0.56% glycogen + 7 ml $NaIO_4$)

$$= \frac{5.5 \times 10^{-5} \times 47}{2} = 1.29 \times 10^{-3} \text{ moles}$$

40 mls 0.5% glycogen

$$= \frac{0.50 \times 40}{100} = 0.20 \text{ g glycogen}$$

Therefore, 1 g of glycogen in control consumes

$$\frac{1.29 \times 10^{-3}}{0.20} = 6.45 \times 10^{-3} \text{ moles of } IO_4^-$$

The number of glucose residues per g glycogen was then calculated as follows:

1 g glycogen consumed 6.41×10^{-3} moles of IO_4^-. Therefore, there are 6.41×10^{-3} moles of glucose residue/g of glycogen, since 1 mole IO_4^- attacks 1 glucose residue only.

Theoretically, 162 g of glucose give one glucose residue. Therefore, one g glycogen

$$= \frac{1}{162} = 6.2 \times 10^{-3} \text{ moles of glucose residue}$$

Similarly, for the control sample:

Since 1 g of glycogen consumed 6.45×10^{-3} moles of glucose, therefore, it has 6.45×10^{-3} moles of glucose residues.

APPENDIX D (Continued)

(b) Production of Formic Acid

(Uremic Sample)

The number of moles of HCOOH produced from 10 ml aliquots of 50 ml samples

$= (0.30 \sim 0.05)$ or $0.25 \times 0.05 \times 10^{-3}$ moles (corrected for blank) (since 0.05 N NaOH was used).

$$= 1.25 \times 10^{-5} \text{ moles}$$

Therefore, total number of moles in 50 ml

$$= \frac{1.25 \times 10^{-5}}{10} \times 50 = 6.25 \times 10^{-5} \text{ moles}$$

This is in 0.184 g of glycogen; therefore, 1 g of glycogen produces:

$$\frac{6.25 \times 10^{-5}}{0.185} = 3.4 \times 10^{-4} \text{ moles}$$

Therefore, 1 g of glycogen $\equiv 3.4 \times 10^{-4}$ moles of non-reducing ends. (Since number of moles of HCOOH formed = number of moles of non-reducing ends).

Similarly, in the control:

10 ml produced $(0.20 \sim 0.05)$ or $0.15 \times 0.05 \times 10^{-3}$ moles

(correct for blank)

$$= 0.75 \times 10^{-5} \text{ moles of HCOOH.}$$

APPENDIX D (Continued)

Therefore, the total number of moles of HCOOH in 50 ml

$$\frac{0.75 \times 10^{-5} \times 50}{10} = 3.75 \times 10^{-5} \text{ moles}$$

This is in 0.20 g of glycogen, therefore, 1 g of glycogen

$$= \frac{3.75 \times 10^{-5}}{0.20} = 1.88 \times 10^{-4} \text{ moles of non-}$$

reducing end.

(c) The Determination of Reducing Ends (Uremic Sample)

The following is the description of the set-up of standard glucose curve, glycogen samples (both for uremic and control) and the results obtained after reading OD at 540 nm (see page 333). The corresponding glucose quantity was interpolated for each sample from the standard curve in figure 26.

The number of moles of reducing ends per g of glycogen by the dinitrosalicylate method:

25 mg glycogen ≡ 25.5 x 10^{-6} g reducing group (from graph ≡ 25.5 μg glucose). Therefore, 1 g of glycogen

$$= \frac{25.5 \times 10^{-6}}{25 \times 10^{-3}} = 1.02 \times 10^{-3} \text{ g}$$

DETERMINATION OF REDUCING ENDS BY

3.5-DINITROSALICYCLIC ACID

# Tubes		1	2	3	4	5	6	7	8	9	10
In ml		1.0	0.95	0.90	0.80	0.70	0.55	0.50	0.50	0	0
	Standard glucose 1 mg/ml	0	0.05	0.10	0.20	0.30	0.45	0	0	0	0
Glycogen	U-50 mg/ml	0	0	0	0	0	0	0.50	0	0	0
	U-50 mg/ml	0	0	0	0	0	0	0	0.50	0	0
	C-50 mg/ml	0	0	0	0	0	0	0	0	1.0	
	C-50 mg/ml	0	0	0	0	0	0	0	0	0	1.0
O.D. at 540 nm		0	0.020	0.035	0.095	0.160	0.360	0.013	0.013	0.041	0.041
μg of glucose from the graph (Fig. 26)		0	50	100	200	300	450	25.5	25.5	48.0	48.0

APPENDIX D (Continued)

Therefore, the number of moles of reducing ends per g of glycogen $= \frac{1.02 \times 10^{-3}}{180} = 5.7 \times 10^{-6}$.

Similarly, for control;

$$0.05 \text{ g glycogen} \equiv 48.0 \times 10^{-6} \text{ g reducing ends},$$

therefore, 1 g of glycogen

$$= \frac{48.0 \times 10^{-6}}{0.05} = 9.6 \times 10^{-4}.$$

Therefore, the number of moles of reducing ends per g glycogen

$$= \frac{9.6 \times 10^{-4}}{180} = 5.30 \times 10^{-6}$$

(d) Calculation of Complete Structure

i. The Average Number of Glucose Residues per Mole of Glycogen

Since the number of reducing ends = the number of moles of glycogen, the average number of glucose residues per mole of glycogen

$$= \frac{\text{no. of residues of glucose/g}}{\text{no. of reducing ends/g}}$$

$$= \frac{6.41 \times 10^{-3}}{5.7 \times 10^{-6}} = 1125 \text{ (uremic)}$$

APPENDIX D (Continued)

$$= \frac{6.45 \times 10^{-3}}{5.3 \times 10^{-6}} = 1217 \text{ (control)}$$

ii. The Average Molecular Weight = Residue M. Wt. x No. of Glucose Residues/Mole

$$= 162 \times 1125 = 1.82 \times 10^{5} \text{ (uremic)}$$

$$= 162 \times 1217 = 1.97 \times 10^{5} \text{ (control}$$

iii. The Average Number of Branching Points = Number of Non-reducing Ends - 1

$$= 60 - 1 = 59 \text{ (uremic)}$$

$$= 36 - 1 = 35 \text{ (control)}$$

Since the number of non-reducing ends/mole of glycogen

$$= \frac{\text{no. of moles of non-reducing ends/g}}{\text{no. of moles of reducing end/g}}$$

For uremic = no. of non-reducing ends

$$= \frac{3.4 \times 10^{4}}{5.7 \times 10^{-6}} = 60$$

For control = no. of non-reducing ends

$$= \frac{1.88 \times 10^{-4}}{5.2 \times 10^{-6}} = 36$$

APPENDIX D (Continued)

iv. Average Number of Segments

$= 2 \times \text{no. of non-reducing ends} - 1$

$= 2 \times 60 - 1 = 119$ (uremic)

$= 2 \times 36 - 1 = 71$ (control)

v. Average Number of Glucose Residues per Segment

$$= \frac{\text{Av. glucose residue/mole}}{\text{Av. no. of segments}}$$

$$= \frac{1125}{119} = 9.5 \text{ (uremic)}$$

$$= \frac{1217}{71} = 17.1 \text{ (control)}$$

3. Glucose Calculation

To calculate the glucose concentration, a series of standards were run and the value for F was calculated in the following way, and was found to be 463.

$$F = \frac{\text{mg\% glucose standard}}{\Delta A \text{ of the standard}}$$

For unknown samples, the glucose concentration was obtained by calculating ΔA for the sample and multiplying it by the factor (F = 463) derived in the above equation.

Mg glucose (per 100 ml plasma) of unknown

$= F \times \Delta A$ of unknown.

APPENDIX E

DETERMINATION OF PLASMA FREE AMINO ACIDS

	Column No. 1 (Short Column)		Column No. 2 (Long Column)	
Type of resin	Resin		9% DVB (divinyl benzene) 12 microns size	
Height of resin	0.8 x 25 cm		0.8 x 50 cm	
Flow rate: Buffer pump	0.8 ml/min		0.8 ml/minute	
Column back pressure	12 Kg/cm^2		26 Kg/cm^2	
1st temperature	45°C		45°C for 2-1/2 hours	
2nd temperature	55°C		55°C for remaining time	
Temperature change time	190 min		150 minutes	
Reaction bath temperature			95°C	
Range	570 mμ x 3		440 mμ x 3	
Mobile phase	Sodium citrate buffer		Lithium citrate buffer	
	pH	Flow rate (min)	pH	Flow rate (min)
1st buffer	4.15	210	2.62	120 for 2 hours
2nd buffer	5.36	155	3.00	120 for 2 hours
3rd buffer			3.90	180 for 2-1/2 hours

Amino acid standard (physiological) A/N used: Each ml of solution contained 2.5 micromoles of each amino acid except

APPENDIX E (Continued)

as indicated in parenthesis, in 0.1 N HCl.

L-Asparagine (1.25)
L-α-amino-*n*-butyric acid
DL-O-phosphoserine (1.25)
L-cystathionine (1.25)
Hydroxy-L-proline
L-glutamic acid
L-methionine
L-tyrosine
L-cystine (1.25)
L-phenylalanine
L-proline
L-alanine
L-threonine
L-serine
L-α-amino adipic acid (0.62)
DL-β-aminoisobutyric acid
O-phosphoethanolamine (1.25)
L-citrulline (0.62)
L-sarcosine (1.25)
Taurine (1.25)
L-aspartic acid
L-isoleucine
Glycine
L-valine
L-leucine
Urea (37.50)
β-alanine (1.25)

20087 Amino acid standard physiological B. Each ml of solution contains 2.50 micromoles of each amino acid and ammonia except as indicated in parenthesis, in 0.1 N HCl.

DL plus allo-delta hydroxylysine
Ammonia as $(NH_4)_2SO_4$
L-tryptophan
L-1-methylhistidine
L-histidine
L-arginine
L-lysine
γ-aminobutyric acid
L-ornithine
L-3-methylhistidine
L-anserine (1.25)
L-carnosine

APPENDIX E (Continued)

Dilution Factor

For example: three ml of plasma after deproteinization gave 1.8 ml supernatant. Then 0.7 ml internal standard was added to give a T.V. = 2.5 ml (pH adjusted to 2.5).

Dilution factor for μM per ml

$$= \frac{0.8 \times 1.8}{2.5} = 0.576 \text{ mls. plasma On the column}$$

Therefore, dilution factor

$$= \frac{0.08}{0.576} = 0.1389.$$

APPENDIX F

Restricted Diet

The consumption of food by uremic rats was followed over a period of 4 weeks. It was observed that the average daily food intake in the uremic group was not more than 23 g per rat (range being 20-23 g per rat per day) compared to the Sham operated control group, whose daily food intake ranged between 28 and 32 g per rat. Thus, in the uremic group the food intake decreased by about 30% in comparison with the normal.

To delineate the effect of caloric deficiency or decreased food intake, secondary to uremia, from the total uremic effect on different parameters measured the diet intake was restricted in a group of rats; those rats were maintained by giving each of them only 23 g daily of the same food as given to the sub-totally nephrectomized rats and the sham operated control rats.

CHAPTER IX

CURRICULUM VITAE

NAME: Abdul Mannan

PLACE OF BIRTH: Sayedpur, Dacca, Bangladesh

YEAR OF BIRTH: 1932

POST-SECONDARY EDUCATION AND DEGREES:

Salim Ullah Muslim Hall
University of Dacca
Dacca, Bangladesh
1950 - 1954
B.Sc. Hons. (Chem.)

Salim Ullah Muslim Hall
University of Dacca
Dacca, Bangladesh
1954 - 1955
M.Sc. (Bio) (Thesis Group)

Faculty of Pharmacy
University of Toronto
Toronto, Ontario, Canada
1968 - 1971
M.Sc. (Pharmacy)

Faculty of Pharmacy and
Pharmaceutical Sciences
University of Alberta
Edmonton, Alberta, Canada
1971 - 1973
Ph.D. (Bionucleonics)
(Thesis Submitted)

HONOURS AND AWARDS:

Colombo Plan Research Fellowship
with the Fisheries Research Board
of Canada
1958 - 1960

Colombo Plan Scholarship
(Post-Graduate Studies)
with the Faculty of Pharmacy
University of Toronto
1968 - 1971

HONOURS AND AWARDS (Continued):

Myrtle B. Field Estate Award
University of Toronto
1971

Graduate Teaching Assistantship
Faculty of Pharmacy and
Pharmaceutical Sciences
University of Alberta

RELATED WORK EXPERIENCE:

(1) Assistant Technologist
Fisheries Res. Lab.
Comilla, Bangladesh
1955 - 1958

(2) Technical Information Officer
Dacca, Bangladesh
1960 - 1962

(3) Research Officer
(Food and Nutrition) CSIR
Dacca, Bangladesh
1962 - 1963

(4) Assistant Professor
(Biochemistry) since 1963
University of Dacca
Dacca, Bangladesh

(5) House Tutor, Iqbal Hall
(Now, Sergent Zahirul Hoq Hall)
University of Dacca
Dacca, Bangladesh
1967 - 1968

(6) Ex-member, Central Board
for Development of Bengali
(Biochemistry and Nutrition
Section)
Dacca, Bangladesh
1963 - 1968

(7) Professional Member
Chemical Institute of Canada
1959

RELATED WORK EXPERIENCE (Continued):

(8) Associate Editor
Journal of Biological and
Agricultural Sciences,
jointly published by the
University of Dacca and
Agriculture University of
Mymensingh
1964 - 1968

(9) Founder General Secretary
Nutrition Society
University of Dacca
1966 - 1968

(10) Member, Academic Council
University of Dacca
1966 - 1968

(11) Demonstrator in Pharmacognosy
(part-time)
University of Toronto
(Faculty of Pharmacy)
October 1, 1970 - May 31, 1971

(12) Graduate Teaching Assistant
Faculty of Pharmacy and
Pharmaceutical Sciences
University of Alberta
Edmonton, Alberta, Canada
September, 1971 - December, 1973

(13) Colombo Plan Research Fellow
Atlantic Fisheries Res. Lab.
at Halifax, Nova Scotia
1958 - 1959

(14) Colombo Plan Research Fellow
Pacific Fisheries Res. Lab.
at Vancouver, British Columbia,
and Freshwater Fisheries Res. Lab.
at Grand Reverie, Gaspe, Quebec
1959 - 1960

PUBLICATIONS:

1. Preparation of deoxynucleosides, purine, and pyrimidine bases and deoxyribose-I-phosphate from milt DNA employing salmon enzyme system: J. Fish. Res. BD. Canada 18(3), 1961, pp. 349-366 (A. Mannan & H.L. TARR)

2. Proximate composition of Canadian Atlantic Fish (I); Variation in composition of different sections of flesh of Halibat: ibid., 18(4), pp. 483-493; 1961. (A. Mannan & W.J. Dyer).

3. Proximate composition of Canadian Atlantic Fish (II): Mackerel, Tuna & Sword fish: ibid., 18(4), 1961, pp. 495-499 (A. Mannan & W.J. Dyer).

4. Proximate composition of Canadian Atlantic Fish (III): Sectional differences in the flesh of Chondrostie, one of chimeerae and some of miscellaneous teleosts: ibid, 18(6), 1961, pp. 893-905. (A Mannan & W.J. Dyer).

5. Preparation of certain fishery specialty products from fish waste and waste fish of E. Pakistan freshwater fisheries: (I) FISH-roe product: Cyprinus cavier: Pak. J. Biol. Agric. Sciences 6(I), 1963, pp. 8-18, (A. Mannan).

6. Preparation of certain fishery specialty products from fish waste and waste fish of E. Pakistan freshwater fisheries: (II): "sidal", an autolysed and semi fermented product: ibid, 6(2), 1963, pp. 70-75 (A. Mannan).

7. Partition of protein and non-proteinous constituents in different parts of fish body: Scientific Researches, 1(3), 1964, pp. 180-186, (A. Mannan & M.Q. Khuda).

8. Production of Food-Yeast on molasses, waterhyacinth and jute stick hydrolysates and palmjuice of E. Pakistan origin: Pak. J. Biol. and Agric. Sci., Vol. (2), 1965, pp. 240-254, (A. Mannan & K.Ahmad).

9. Distribution of unsaponifiables in oils of E. Pakistan food-stuffs: Pak. J. Biol Agric. Sci., 9(1), 1966, pp. 30-32 (A. Mannan & K. Ahmad).

10. Studies on vitamin E in food-stuffs of E. Pakistan: Pak. J. Biol. Agric. Sci., 9(1), 1966, pp. 13-19, (A. Mannan & K. Ahmad).

11. Fatty acid (FA) patterns of some common fats and oils by GLC: Proc. 18th-19th All Pakistan Science Conference: Section, Chemistry C-52 (III), 1965, (A. Mannan & K. Ahmad).

12. Studies on fatty acid patterns of adipose tissue lipid in marasmus patients: ibid, C-53(iv), 1965, (A. Mannan & K. Ahmad).

13. Fatty acid patterns of blood lipid in marasmus patients by GLC: ibid, C-53(v), 1965 (A. Mannan & K. Ahmad).

14. Studies of Fatty acid patterns of blood lipid obtained from obstructive jaundice patients: ibid, C-54(vii), 1965, (A. Mannan & K. AHmad).

15. Fatty acid patterns of phospholipid, cholesterolester, and glycerides fractions of plasma lipid from jaundice patients: ibid, C-54(viii), 1965.

16. Fatty acid patterns of blood plasma from T.B. patients: ibid, C-54, 1965, (A. Mannan & K. Ahmad).

17. Effect of local edible oils on fatty acid pattern as well as lipid class lipids of rat tissue: ibid, C-41-43, 1965:

18. Effect of carbon tetrachloride on fatty acid pattern of tissue lipids in rats: one student did his M.Sc thesis.

19. Blood constituents and fatty acid patterns of untreated Diabetes Melletus: Proc. 21st All Pakistan Diabetic Association held in Dacca in April, 1968, (A. Mannan & K. Ahmad).

20. New Sources of Proteins, presented before 1st Nutrition Conference held in Dacca in June, 1968 under the auspices of the Nutrition Society at Dacca University. (A Mannan).

21. Norcholane & Cholestane Analogues of Cucurbitacin. 1. Synthesis & Inhibition of DNA Synthesis in Rat Thymocytes. Chemical Communications, 1971, Comm. 654, April 27th, London, (A. Mannan & G.R. Ducan).

22. Experimentally Induced Chronic Uremia in Rats. The Influence on Carbohydrate Metabolism. Presented in 'BioPharmacy' section at the Nineteenth Canadian Conference on Pharmaceutical Research, University of Alberta, Saturday, August 12th, 1972. (A. Mannan and L.I. Wiebe *et al*).

23. Certain Metabolic Aspects and Clinical Evaluation of Experimentally Induced Chronic Uremia in Rats. (A. Mannan and L.I. Wiebe *et al*). (Submitted for publication). (1973).

24. Thymus and Thymocyte response to some novel steroids (Mannan et al and G.R. Duncan). Presented in 20th Canadian Conference on Pharmaceutical Research, Dalhousie University, Halifax, N.S., May 4-5 (1973), #6.

25. M.Sc. (Biochemistry) Thesis on "Production of Food Yeast on Molasses, Waterhyacinth and Jute-stick Hydrolysates". University of Dacca (Dept. of Biochemistry and Nutrition). Ramna, Dacca, Bangladesh, 1955.

26. M.Sc. (Pharmacy) Thesis on "The Effect of Glucocorticoids and Analogues on Nucleoside Uptake into Rat Thymocytes". University of Toronto (Faculty of Pharmacy), Ontario, Canada, 1971.

27. Ph.D. (Bionucleonics) Thesis on "The Influence of Sub-Total Nephrectomy on Glucose Metabolism in Rats". University of Alberta (Faculty of Pharmacy and Pharmaceutical Sciences), Edmonton, Canada, 1973.